The role of arbuscular mycorrhizal fungi in the early growth of rowan (Sorbus aucuparia L.)

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ABSTRACT

An extensive literature search indicated that many trees on landscape sites fail to establish because of poor initial plant quality and sub-optimal planting conditions. Arbuscular mycorrhizal fungi (AMF) form a symbiotic association with many landscape plants, with the potential to improve plant establishment through improvements to root structure and enhanced stress tolerance. Several experimental studies were undertaken to determine whether rowan (*Sorbus aucuparia* L.), a stress-tolerant native tree, formed associations with AMF, and whether this lead to improved early growth and field performance.

A field study indicated that rowan did form associations with AMF and that root colonisation levels of 30-40% were typical on nurseries and sites. The use of soil as an inoculum was investigated, and suggested that under poor nutrient conditions, micro-organisms including AMF improved growth, but that under higher nutrient levels these same organisms inhibited growth. Under more standardised conditions, inoculation with commercial forms of *Glomus intradices* Schenk & Smith but not *Glomus mosseae* (Nicol. & Gerd.) Gerdemann & Trappe was found to improve early growth and winter survival. However when inoculated cell-grown plants of rowan, ash (*Fraxinus excelsior* L.) and cherry (*Prunus avium* L.) were transplanted onto a reclaimed oil-shale bing results were not so conclusive. Root mass was increased following inoculation in rowan and ash, but decreased in cherry. Inoculation was also associated with reduced survival in cherry, suggesting that further studies of the field performance of inoculated plants are required. A conceptual decision model to identify situations where inoculation with AMF might be cost-effective and beneficial is presented.

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CHAPTER ONE
INTRODUCTION

"A blessing on the man that puts his trust in the Lord...he is like a tree by the waterside that thrusts its roots to the stream: when the heat comes it feels no alarm, its foliage stays green; it has no worries in a year of drought, and never ceases to bear fruit."

Jer.17 :7-8.

1.1. BACKGROUND TO THE PROBLEM.

How is tree quality affected by mycorrhiza? This thesis is an attempt to explore interactions between mycorrhiza and seed provenance, and how they may be applied in a cost-effective and sustainable manner, to the practical problems of poor tree survival and performance on urban landscape sites.

A number of studies have highlighted the problem of poor tree survival on landscape sites, where failure rates between 20 and 50 per cent have been reported. Expenditure on amenity tree planting was estimated at £60 million in 1991 [1] and has no doubt increased since then. This suggests that tree failure has serious financial implications for the landscape profession, while also reducing public interest and support for urban tree planting schemes. Despite much research into identifying the causes of tree failure, trees continue to ail and die on many landscape planting schemes.

Urban landscape sites have by definition been altered by urbanisation processes: construction, industrialisation, and in some cases post-industrial dereliction. Therefore a combination of soil and climatic conditions exists for plant growth which differ considerably from those under which many amenity plants evolved. In addition to these site stress factors, landscapers often specify the cheapest plant material, despite

awareness of the importance of plant quality to successful plant establishment, and then fail to maintain planting schemes to an adequate standard.

The market for native trees has shown enormous potential in the last few years as public funding has been directed towards woodland projects that have social and environmental benefits. Native trees are often planted for nature conservation, multi-purpose forestry and their visual appropriateness in the landscape. When the Government tax incentive scheme for forestry was abolished in 1988, the market for conifers transplants plummeted. Many tree growers switched production from forestry conifers to native trees which appeared to have a more buoyant market. Incentives such as the Woodland Grant Scheme encouraged woodland creation on set-aside land, with broad-leaved species attracting a higher level of grant than conifers. By 1994/95, more broad-leaved trees were being planted (12,600 ha) than conifers (11,700 ha) [2]. It was also anticipated that projects such as the Millennium Forest for Scotland which received funding from the Lottery Commission would stimulate demand for native trees. Despite this initial optimism, in many cases the increased demand failed to materialize, and many nurseries have faced severe financial difficulties, leading to a need to reduce costs, or raise prices by offering a higher quality product

1.2. DEFINITION OF TERMS USED IN THE THESIS

Plant quality is often used to predict plant performance following the transplanting process. Various definitions of planting stock quality have been proposed, encompassing morphological and physiological attributes of plant condition, either when it leaves the nursery or at the time of planting. At present no definition includes genotypic suitability or biological criteria, such as the presence of root symbionts.

The term mycorrhiza (literally 'fungus-root') refers to a symbiotic association between plant roots and ubiquitous soil fungi. There are several types of mycorrhiza, but the most important to the landscape industry are the arbuscular mycorrhizal fungi (AMF), which associate with a greater range of plant species. AMF are an inexpensive and sustainable form of biotechnology, with demonstrable effects on plant quality.

Seed provenance, refers only to the geographic location from which seed is collected. It gives no indication of origin, that is, whether the mother plant was locally indigenous to that area. The term is often interpreted within the context of genotypic variation, but this is not implicit in its definition. In this study although some consideration will be given to the potential to exploit seed provenance to improve planting stock quality, in the experimental section, seed provenance will only be considered as an interactive factor with AMF.

Native trees are those that arrived "unaided by man's efforts" since the last Ice Age, while Britain was still connected by a land bridge to the rest of Europe [3]. Of these, Soutar [4] has compiled a list of trees native to particular zones in Scotland, noting that native species are usually indigenous to particular zones, rather than to the country as a whole, citing the example of Scots Pine (*Pinus sylvestris*) which is only really native to the Highlands. The main Scottish native trees, preferred site conditions, life strategy and mycorrhizal association are given in Appendix 1. This was used as the basis of tree species selection in the field experiment.

Sustainability has been defined as "...meeting the needs of the present without compromising the ability of future generations to meet their own needs" [5]. Within the context of this study, sustainability is interpreted as the responsible use of non-

renewable resources (including growing substrates, fertilisers, biocides and fossil fuels) in plant production and landscape management.

The rowan (*Sorbus aucuparia* L.) is an attractive, native tree often planted on derelict sites, owing to its hardiness and ability to tolerate poor soil conditions. It has been little studied by previous researchers, particularly with regard to the significance of mycorrhizal associations and seed provenance. Rowan was chosen as a case study for this thesis as it is a good model for plant survival under adverse environmental conditions.

1.3. NEED FOR INVESTIGATION

There is a need to address the issue of technology transfer with mycorrhizas in the landscape industry. Until recently, despite several decades of fundamental experimental work on mycorrhizas, there was little awareness or uptake of the technology. However, in the last year or so, interest in the subject has proliferated. This has been associated with developments in the technology of inoculum production and the launch of mycorrhizal and microbial products targeted at the landscape industry by a large US company. Mycorrhizal products have had a higher profile within the trade literature, and many nurseries and local authorities have apparently been persuaded to trial mycorrhizal products. All these factors have helped to increase awareness, such that mycorrhizal fungi appear to have been promoted as a panacea to all the problems associated with landscape sites. However, many of the products offered on the market may be of dubious quality, and few published trials have been conducted scientifically under realistic site and nursery conditions. Therefore, it is necessary to:

- explore the functioning of AMF in different soil conditions

- explore the mycorrhizal responsiveness of a range of plant genotypes (seed provenance, tree species)
- explore the mycorrhizal status of rowan
- demonstrate AMF benefits in a practical landscape context
- explore situations in which it would be cost-effective and beneficial to use AMF
- consider some of the practical implications to nurseries and landscape practitioners in terms of cultural and management practices .

1.4. APPROACH

The current study has been undertaken within the context of landscape architecture and therefore attempts to bridge the gap between forestry/botany and the practical issues pertinent to the subject domain. The approach chosen for the study comprises a literature review; a series of field and experimental studies, and conceptual AMF decision model, based on cost-benefits.

The first chapter of the literature sets the context of the study by exploring the problem of poor tree survival. This is followed by an exploration of how this might be remedied by improving planting stock quality at the nursery stage, and particularly rooting structure, by a modification of cultural practices and the use of root symbionts such as AMF. The second chapter considers the use of arbuscular mycorrhizal fungi, and presents experimental evidence of beneficial effects on tree growth. The ecology of the organisms that form the association, as well as practical aspects of their management is also discussed. The third chapter proposes rowan as a model of plant survival on hostile sites, and discusses its use in the landscape, ecology and evidence of genetic variation. The literature review concludes by highlighting areas of potential

research, and sets up the research aims and objectives (hypotheses) to be addressed in the experimental section (Chapter Five).

The experimental section begins with a description of the research methodology common to the experimental studies, and includes a review of methods used in the study of arbuscular mycorrhizal fungi (Chapter Six). These will be of importance to nurseries considering adopting mycorrhizal technology. The succeeding experimental studies explore the mycorrhizal status of rowan on nurseries and landscape sites (Chapter Seven), and how its growth and survival are modified by soil factors, including mycorrhizas (Chapter Eight). Chapter Nine presents a glasshouse experiment to consider the effects of inoculation with known strains of mycorrhizal inoculum, and interactions with seed provenance. The final field study (Chapter Ten) is concerned with the field performance of inoculated plants and expands the relevance of the work by including other native landscape tree species, namely ash (*Fraxinus excelsior* L.) and wild cherry (*Prunus avium* L.), and a management practice. The final discussion draws together the experimental findings and applies them to the practical problem of tree quality and performance. The discussion is followed by a conceptual model, which aims to identify situations in which it might be cost-effective and beneficial to manage AMF in the landscape.

CHAPTER TWO
TREE SURVIVAL ON LANDSCAPE SITES

2.1. INTRODUCTION

Since the 1980's, a number of studies have highlighted the problem of poor tree survival and performance on urban landscape sites. Mortality rates of between 20 and 50% have been reported, depending on site conditions, choice of tree species and supplier (Table 2.1). In addition to the financial costs of tree replacement - annual expenditure on amenity trees in 1991 was estimated at £60 million [1] - high failure rates may also erode public support for tree planting schemes. Since 1980, much research has been commissioned to investigate the causes of poor establishment and transplant stress in newly planted trees.

Table 2.1. Survival studies in newly planted trees.

Authority	Mortality Rate	Study site
Capel [6]	0-85% after 5 years, depending on site	Range of 119 urban sites across UK
Insley [7]	20% after 1 year	Forestry Commission transplants on motorway embankments
Skinner [8]	46% after 5 years	Standard trees on land renewal schemes in Central Scotland
Kendle *et al.*[9]	12 % for beech 34% for birch (including 86% from one particular supplier)	Experimental trial to compare performance under good planting conditions, of transplants from range of suppliers
Gilbertson & Bradshaw [10]	23% after 3 years, further 16% in next 2 years	Inner city site in Liverpool
Hodge [11]	27% (after 7 years) for oak half standards 39% for whips and 90% for transplants. Rowan 75% regardless of stock type.	Motorway embankment on disturbed clay soil. No weed control.

In this chapter the main causes of poor tree survival and performance on urban landscape sites are reviewed. A definition of planting stock quality is introduced,

followed by a discussion of the importance of rooting structure in newly-planted trees. Consideration is given to how planting stock quality may be manipulated by cultural practices and the selection of suitable seed provenances at the nursery stage.

2.2. CAUSES OF POOR TREE SURVIVAL

Drought is one of the major causes of death in newly planted trees, resulting from the inability of the root system to supply adequate moisture to the establishing tree [9, 12]. This may be related to poor quality planting stock, root damage prior to planting, site stress factors, inadequate site management and human factors. Gilbertson and Bradshaw [10] observed that tree mortality tended to peak at the time of planting and also at bud burst in both the first and second season following transplanting.

Poor quality planting stock can result from factors controlled at the production stage. Growing conditions particularly nutrition and irrigation, will affect size, the ratio of roots to shoots and also the plants carbohydrate reserves required to maintain the plant during the establishment phase. Insley [13] observed that the plants of *Acer pseudoplatanus* that survived transplanting tended to be larger than those which died. However Kendle *et al.*[9] failed to establish a simple relationship between size and transplant survival. Physiological factors such as dormancy status also affect subsequent performance - bare rooted stock lifted before the onset of full dormancy will be less resistant to frost and desiccation damage [14]. The use of sterile growing media on nurseries and use of fertilisers and pesticides may also discourage root symbiotic organisms such as mycorrhizal fungi and nitrogen-fixing bacteria.

Damage also occurs to stock during handling stage between lifting in the nursery and actual planting on site. Bare rooted trees lose a significant proportion of their root

system at lifting: Watson [15] estimated that up to 95% of the root system may be lost when the tree is lifted in the nursery. Root losses result in a loss of stored carbohydrate, and an imbalance between the root and shoot system resulting in severe water stress. Further root loss may occur due to root desiccation caused by exposure. Insley [13] observed a direct relationship between survival and moisture content at planting for a range of broad-leaved trees which was only partially relieved by re-wetting. More detailed studies involving *Fraxinus* and *Betula* [16] indicated differences between the species and root types in the rate of drying out, as well as desiccation tolerance. Lateral roots tended to die within 24 hours, resulting in a loss of up to 39% in *Betula* compared with between 26-29% in *Fraxinus.* Similarly, Dutton and Bradshaw [17] recorded an almost ten-fold difference in survival between rowan (68%) compared to birch (8%) following 7 days storage without protection, compared to 83% survival in both species when the roots were stored in polythene bags. Root loss and desiccation also damages the root symbionts which may have be present within the root system.

The stress factors to which plants on certain urban sites are exposed have been reviewed by Craul [18]; Bradshaw *et al.,* [12] and Kendle [19]. These stress factors include loss of soil structure resulting in compaction; extremes of pH; deoxygenation caused by waterlogging or landfill gases such as methane; nutrient deficiency; mineral or contaminant toxicity. Soils may also be extremely variable, containing building rubble and other waste materials, adding to the difficulty in correcting soil problems, and tend to be deficient in beneficial soil micro-organisms. Soil compaction often occurs during the construction phase and may be relieved by ground cultivations. Severe compaction will reduce root growth: no root growth usually occurs above bulk densities of 1.6 mg m^{-3} for clay soils and 1.8 mg m^{-3} for sandy soils [20].

Post-planting, additional stress factors on landscape trees are associated with inadequate maintenance, particularly weed control, and other human factors [12]. Competition from weed species for light, water and soil nutrients create additional stresses for the establishing plant. Stem damage results in loss of cambial tissue and the entry of pathogens. It has several causes: poorly maintained or inappropriate stakes, tree ties and tree guards, as well as mowers and strimmers resulting in stem. Finally there are the effects of vandalism, perceived as the main threat to urban trees, but which may be exacerbated by tree location and size of planting stock [8, 12].

2.3. PLANTING STOCK QUALITY

Planting stock quality has been defined as 'fitness to purpose', that is the extent to which ' ...stock realises the objectives of management ... at minimum cost' [21]. Kendle *et al.* [9] suggested that stock quality encompassed a more complex association of attributes determined at the production stage which should ensure that the transplant was free from damage, able to survive the transplanting process and capable of rapid root expansion. The quality of bare-rooted forestry trees is covered by British Standard 3936 [22] which sets out minimum height and root collar diameters for a range of species. It states that the root system should be capable of establishment and in proportion to stem height, and that plants show no signs of mechanical damage, discoloration due to mineral deficiency or toxicity, and are free from pests and diseases. This standard has been criticised as it relies on external morphological features which may not adequately predict survival and performance after transplanting, and takes no account of physiology or biological fitness [23, 24].

Various physiological measures have been proposed to address the problem of identifying poor quality planting stock prior to planting [24, 25, 26]. These include viability staining techniques [27, 28, 29]; root growth potential [30] and estimates of cell membrane integrity [2]. Cell viability stains such as 2,3,5-triphenyl tetrazolium chloride (TTC), which is widely used to assess seed viability, has been used with mixed success on a range of broad-leaved tree species. The principle of the test is the reduction of TTC from a colourless state to red formazan by the dehydrogenase enzymes present in the mitochondria of living cells. Root desiccation experiments with *Acer platanoides* indicated that formazan production decreased by 50% after 96 hours desiccation [29]. However more work was required to calibrate the test for different ages and species of root samples, as well as dormancy phase. Root growth potential (RGP) is a measure of a seedling's ability to regenerate roots under standard conditions and has been extensively used on forestry transplants. Cell electrolyte leakage assesses the damage to cell membranes by measuring changes in the conductivity of a solution containing a sample of root tissues: the more damaged the tissue the greater the leakage of cell materials.

Since 1992, the Forestry Commission has offered a chargeable plant quality testing service to tree producers. Six quality indicators based on physiological and morphological criteria, have been defined for a range of broad-leaved species including rowan, oak, birch and beech [2]. These indicators are given in Table 2.2, which also gives the threshold values for rowan and birch, which should result in at least 80% survival, given reasonable site conditions and early maintenance. Field trials across a range of urban sites in the UK indicated that cell electrolyte leakage was the best predictor of survival. Threshold values ranged from 22 % for rowan, down to 17.7 % for birch. Where ecto-mycorrhizal fungi were detected on the roots, the

threshold value may be raised by 5% (Colin Edwards, pers. comm.). The sturdiness quotient, based on the ideal stem diameter for a given height, was adapted from BS3936 Part 4, which does not mention rowan. The best predictor of first year height in rowan was shoot moisture content; for birch it was the sturdiness quotient.

Table 2.2. Plant quality indices and threshold values for young rowan and birch trees (From: McKay [2]; McKay and McEvoy [26]).

Attribute	Significance	Measure	Threshold Values (%)*	
			Rowan	Birch
Large woody root** condition	Best predictor of potential performance and survival	Cell electrolyte leakage (%)	<22.0	<17.5
Quantity of large woody roots	Indicates stored reserves of water, carbohydrate and nutrients	Dry weight (g)	-	>1.0 g
Sturdiness quotient	Ratio water supplying tissue (roots) to water losing tissue (shoots)	$\frac{\text{Height(cm)}}{\text{Diameter(mm)}}$	-	<12
Fine root*** condition	Root regeneration and water uptake	Cell electrolyte leakage (%)	<16	-
Quantity of fine and lateral roots	Correlates with stem growth in first year after growth	Dry weight (g)	-	-
Shoot moisture content	Initial water status. Good predictor of first year height	$\left(\frac{\text{Fresh Weight} - \text{DryWeight}}{\text{Dry Weight}}\right) \times 100$	>56	-

* Levels at which 80 % post-planting survival may be expected.
** Large woody roots are greater than 5 mm diameter.
*** Fine roots show no signs of secondary thickening and are less than 2 mm in diameter.

2.4. SIGNIFICANCE OF ROOTING STRUCTURE

The main functions of root systems are resource acquisition, carbohydrate storage and plant anchorage. All these functions are of importance during plant establishment. Root loss caused by lifting or desiccation damage changes the root to shoot ratio, depletes carbohydrate reserves and removes potential sites for root regeneration. A reduction in root system size creates an imbalance between the evaporative demands of the shoots and the ability of the roots to absorb water from the soil. Rapid root

regeneration and exploration of the soil environment are therefore essential. These attributes are determined by the physiological status, and architectural configuration of the root system.

A high root growth potential (RGP) reflects the regenerative capacity of the root system and is a good measure of seedling vigour and survival after planting [30]. It is measured by counting the number of new white roots produced on stripped roots that have been grown under standard environmental conditions. RGP indicates the physiological status of the root system, and is controlled by bud dormancy and carbohydrate reserves, mediated by the levels of endogenous hormones. It can be modified by cultural practices in the nursery and subsequent handling. Fibrous-rooted species such as rowan, ash and sycamore (*Acer pseudoplatanus*) tend to transplant easily as they have a high RGP, whereas coarse-rooted species such as oak and hornbeam (*Carpinus betulus*) regenerate roots more slowly through adventitious means and are therefore more difficult to transplant [11, 31]. Birch is an exception in that it is both fibrous-rooted and difficult to transplant: most of the carbohydrate necessary for early growth in birch is stored in the fine roots which are easily damaged by desiccation [32].

Some researchers have observed a relationship between the number of permanent first order lateral roots and field performance in a range of hardwood species. Kormanik [33] observed that the number of lower order roots was a better predictor of planting survival compared to the more conventional measures of stem height and root collar diameter in *Liquidamber, Fraxinus* and *Quercus*. A similar relationship was noted by Thompson and Shultz [34] in red oak (*Quercus rubra*). First order laterals originate from the primary root shortly after germination and unless damaged, will determine the

structure of the mature root system. First order laterals are more robust than higher order roots, and therefore tend to be more resistant to rough handling and exposure. They are important to the establishing plant for water and nutrient uptake, and to provide sites for further root initiation [34]. The number of first order laterals is genetically determined and not affected by nursery growing conditions such as bed density, moisture or fertiliser status or mycorrhizal colonisation [33].

Fitter [35] suggested that ".. for any given set of soil conditions and plant demands, there must be an optimum distribution of roots in the soil and that distribution will be achieved by a particular architectural configuration". It may therefore be possible to select and design root system configurations for particular habitat [36, 37, 38]. Lavender *et al.,* [36] observed that root characters across a range of 30 birch clones were more variable that shoot characters. The clones demonstrated considerable variation in the relative proportions of white, woody and fine roots, specific root length and root mass. These root system attributes reflect differences in carbon allocation within the plant, and would have consequences for the plants' response to soil resources and mycorrhizas.

It has been suggested that root system characters determine the competitive strategy on plants in the field [38, 39]. The relative efficiency with which particular root configurations are able to obtain soil resources, especially those in short supply, has been associated with their construction and maintenance costs. Roots may consume between 50 and 80% of total plant photosynthate, depending on root volume, length, diameter and branching [34, 40]. Fitter [34] proposed two extremes of root topology : herringbone systems which are based on a main root axis and primary laterals, and dichotomous systems where branching may occur along any of the laterals.

Herringbone configurations, being composed of lower order laterals, are more expensive to construct in terms of carbon allocation, but are better at exploring and absorbing soil resources in infertile conditions. In contrast, dichotomous root systems comprise mainly higher order laterals which are cheaper to construct, but rarely explore beyond the zone of depletion, and so typify more fertile habitats.

Atkinson [38] discussed root system design in terms of risk-takers ('competitors') and insurance strategists ('stress-tolerators'). Risk-takers predominate in fertile habitats and allocate fewer carbon reserves to the root system, whereas insurance strategists tend to occur in less fertile habitats and allocate a higher proportion of carbon resources to the root system. Root systems also demonstrate plasticity, that is the ability to modify size and morphology in response to environmental conditions, such as nutrient supply.

Roots and their surrounding rhizosphere are also the site of potentially beneficial associations with nitrogen-fixing bacteria, mycorrhizal fungi, and other soil micro-organisms which have been implicated in disease suppression and the promotion of root symbionts. Mycorrhizal fungi, which will be considered in more detail in the next chapter, have been demonstrated to have a beneficial effect on nutrient uptake [41]; root morphology [42, 43] and root longevity [44]. There is evidence that root system morphology, and the efficiency with which a plant can obtain soil resources, will affect the dependency of the host plant on the mycorrhizal association [45].

2.5. NURSERY PRACTICES TO IMPROVE PLANTING STOCK QUALITY

Inherent in the definition of stock quality proposed by Kendle *et al.* [9] was the assertion that the production and supply process should enhance the ability of the stock to

survive transplanting. A possible conflict in interests between growers and end users was suggested by Bradshaw [46] who argued that production systems should lead to stock of "...high quality performance for the user in both the short and long term, and that are not primarily orientated to benefit growers." The previous section has highlighted the importance of a root system capable of making rapid contact with the planting substrate and obtaining water and nutrients. These properties may be controlled at the production stage by cultural practices which encourage a fibrous root system with a high root growth capacity, manipulation of plant hormones, inoculation with mycorrhizas, and selection of suitable seed provenances / genotypes.

Bare-rooted plants are commonly subjected to lifting, undercutting or root wrenching in the seedbed to encourage a fibrous root system. Richie and Dunlap [30] reported a 10-fold increase in RGP in conifers following root pruning, which acts by removing inhibitors of lateral root emergence in the root tip such as cytokinins and abscissic acid. However the practice also creates a moisture stress in the short term which, depending on the time of application may affect the dormancy status of the plant. Root removal also reduces stored carbohydrate, as well as some of the roots infected by mycorrhizal fungi.

A recent innovation has been to produce trees in small linked cells, such as 'Rootrainers' or 'Rigipots', which are designed to reduce root spiralling. It has been estimated that in 1997 28% of forestry trees (excluding sitka spruce) for sale by Horticultural Trades Association nurseries were cell-grown. This type of production has advantages over bare-rooted stock in that the trees are less prone to root damage from rough handling and root desiccation, retain a greater proportion of intact roots and offer flexibility on planting schedules [11, 12]. Cell-grown trees have a shorter

production period, usually a single growing season, enabling growers to respond more quickly to consumer demand. Production is also more amenable to mechanisation, and there is greater potential to manipulate plant nutrition, pest and disease control as well as inoculate with root symbionts. Cell grown-trees are also amenable to the production of local provenances of native trees, as small batches may be sown, and identified through bar-coding which can be followed on computorised record-keeping procedures. However, cell grown plants tend to be more expensive and may offer no additional benefits in terms of survival, compared to bare-root trees, providing that the plants are carefully handled and planting occurs within the dormant period.[47].

Root regeneration may be encouraged by external application of plant growth regulators. Richardson [48] hypothesised that root growth was controlled by growth factors originating in the shoot. In experimental trials Farmer [49] used auxins to stimulate root growth and inhibit shoot extension in red oak (*Quercus rubra*). Recently, Perceval and Gerritson [50] reported trials in which they applied auxins to a range of containerised trees, including rowan, following removal of 50% of the root system. Root dry weight and root to shoot ratio were increased by a factor of 2-3, although there were differences in the optimum combination and concentration of auxins for each tree species. Although this approach might be used to encourage root regeneration (it originally addressed the problem of root severance following utility trenching), plant growth regulators are sometimes classified as pesticides, and their use is of questionable sustainability.

2.6. SIGNIFICANCE OF SEED PROVENANCE

Survival, growth and environmental adaptation of planting stock may be affected by the geographic location (provenance) and also habitat from which seed is collected [51].

It has therefore been suggested that these differences may be exploited to select trees tolerant of urban landscape sites [52]. Intra-specific variation is generally considered to be a response to habitat selection pressures such as climate, daylength and edaphic conditions. However it is important to separate differences which have a genetic basis from those which may vary in response to particular environmental conditions, a phenomenon known as phenotypic plasticity.

The issue of seed provenance in native trees is a large and contentious subject, beyond the scope of this thesis, and has several important and conflicting aspects: legal (restricted choice of stands as seed sources for forestry species); nature conservation (use of local provenances) and tree selection programmes. Improved selections of tree species are usually propagated as clones and this may be anathema to those who plant native trees for reasons of nature conservation. Clones may also become susceptible to subsequent epidemics of pests and pathogens [53].

The importance of provenance selection is well understood by foresters who require optimal and predictable survival, growth rates and timber qualities. Because of this, the Forest Reproductive Materials Regulations (1977), which have legal status, were set up to control the origin and quality of plant material for a range of species used in forestry. However it does not cover trees grown for 'amenity' purposes. Seed for the species covered by these regulations must be collected from registered stands inspected by the Forestry Commission [54].

The issue of seed provenance has not always been appreciated by landscape architects. Although local provenances are sometimes specified in nature conservation schemes in order to avoid contaminating the native gene pool, many 'native' trees may have been

grown from non-British provenances, or even imported from the continent. This can have devastating consequences for wildlife if the timing of leaf-break and flowering do not coincide with the life-cycle requirements of its associated fauna. There is also evidence that non-local provenances may not be adapted to UK growing conditions. Worrell [51] compared relative growth rate, survival and stem form in British and European provenances of native trees, using data compiled from Forestry Commission trials. In the majority of cases British provenances of oak, pine, birch and alder were superior; however for beech, European sources were superior in 50 % of cases. He linked these finding to the post-glacial history of a tree species: birch (and probably rowan) have been present in Britain for at least 10,000 years, and have had more time to adapt to local conditions, compared to beech which only arrived 2-3,000 years ago. Within the context of Scottish native trees, seed provenance and genetic variation have been widely studied in birch (*Betula* spp.) and used as the basis for tree selection and improvement programmes. Few studies have been carried out in this country with rowan, owing to the lack of commercial interest in the species, although there is some evidence of variation which could be exploited to a greater extent (Section 4.7). Studies have reported intra-specific variation in birch in relation to altitude [55, 56, 57]; ability to form ecto-mycorrhizas [56, 58]; tolerance of heavy metals [56, 58], and root system characters [36]. Some of these trials are summarised below in Table 2.3.

Table 2.3. Provenance trials for *B. pendula* and *B. pubescens*.

	Seed Source	**Trial Conditions**	**Response Parameter**
Habjorg [55]	Norway 56^0N to 69^0N.	Glasshouse, controlled temperature, daylength and light intensity.	Shoot growth and dry matter production
Pelham *et al.* [56]	Scotland 50^0 N, 69^0 N	Field trials at 56^0N and 69^0N	Height, leaf flush, number of mycorrhizas
Good [57]	Norway (59^0N, 950 m) Scotland (58^0N, 46 m).	Field trial	Height, crown shape

Habjorg [55] demonstrated that latitude had an effect on growth responses to photoperiod and light intensity. The southern ecotypes (56^0N) achieved greatest overall shoot dry matter production, although the northern ecotypes (69^0N) achieved their maximum levels at longer daylengths (20 to 24 hours) than the southern ecotypes (18-20 hours). The northern ecotypes were also more efficient at higher light intensities. Pelham *et al.* [56] observed that seedlings of *B. pubescens* performed better when grown at latitudes closest to that from which seed was collected. When grown on a site at 56^0 N, the southerly collections (50^0N) were taller (by a factor of 6) and had a greater stem diameter (by a factor of 4). However when grown at 69^0N, the northerly collections (69^0N) were more vigorous. Leaf emergence for seedlings from latitude 50^0 commenced when days were shorter (14 hours) compared to 16-20 hours for seedlings from latitude N 69^0N. The latitude of seed origin also had a significant effect on the ability of *B. pendula* to form ectomycorrhizal associations with *Hebeloma* spp., *Laccaria* spp. and *Inocybe* spp, suggesting a host-genotype interaction. Southerly trees were associated with 384 fruit-bodies per tree, compared to nil fruit-bodies for the northerly trees. Good [57] observed that populations of *B. pubescens* grown from seed collected in Scotland were taller (data not given), and maintained a narrow crown compared to the Norwegian trees which had a more open form, indicating a loss of apical dominance. Other studies have indicated populations differences in leaf shape, which would have consequences for photosynthesis and transpiration [59]; responses to phosphate levels [59]; zinc tolerance [56]; adaptation to coal spoil heaps [57] and resistance to the rust fungus *Melampsoridium betulinum* [56].

2.7. SUMMARY

This chapter sets the context of the present study, and explores some of the problems of poor tree survival on landscape sites. Successful tree establishment is dependent

upon the quality of plant material at the time of transplanting and also its innate fitness to tolerate the range of environmental conditions and stresses that may prevail on site. Quality is often defined in morphological terms such as height, stem diameter and root to shoot ratio, and increasingly in physiological assessments of root growth potential and cell electrolyte leakage. However any definition of plant quality and fitness should also take account of genetic variation which will affect the form of the root system and inherent adaptation to site conditions. Mycorrhizal associations also have the potential to modify the form and function of roots systems, enhancing competitive strategy and tolerance of adverse site stresses. Neither of these issues (seed provenance or mycorrhizas) are covered by any current definition of planting stock quality. These topics will be explored in the following chapters.

CHAPTER THREE.
ARBUSCULAR MYCORRHIZAL FUNGI.

3.1. INTRODUCTION

The term mycorrhiza (literally fungus-root) refers to a symbiotic association between soil fungi and plant roots, which encompasses a entire range of effects from mutualism to parasitism [60, 61]. The thread-like mycelial body of the fungus can be regarded as intermediates between plant roots and the soil environment. Mycorrhizas have the potential to improve tree growth in the nursery as well as survival and fitness in the landscape. Most benefits attributed to the association have focussed on improvement to nutrient uptake, stress-tolerance and rooting structure. The mycelial network is also thought to contribute to plant community diversity [62]; plant succession [63]; nutrient cycling [64] and soil structure [65].

3.1. BIOLOGY OF MYCORRHIZAS

Two types are mycorrhizal associations are commonly formed with trees: ectomycorrhizas (ECM) and arbuscular mycorrhizas (AM). ECM are distinguished by an external fungal sheath around the root of the host plant and the Hartig net, a fan-like network of hyphae penetrating the epidermis and cortex of the host root. ECM only occur with woody perennials, particularly important forestry species such as *Pinus, Quercus, Fagus* and *Betula*. Following colonisation by ECM fungi the roots become finger-like in appearance. They are attributable to basidiomycetes such as *Rhizopogon, Hebeloma, Amonita, Boletus* and *Pisolinthus,* as well as imperfect fungi such as the ubiquitous *Cenococcum* [66, 67]. Ectomycorrhizal fungi depend on simple monosaccharides and oligosaccharides as carbon sources and are capable of hydrolysing and mobilising sources of phosphate and nitrogen not usually available to plants. They are rarely found as free-living organisms. Many have been isolated and grown in pure

culture – inocula for inoculating seedlings of a limited number of tree species are commercially available. ECM are considered to be expensive to the host in terms of carbon allocation, consuming up to 30% of the carbon compounds translocated below ground [45]. Nevertheless they can greatly aid the growth of seedlings by facilitating nutrient uptake: 3.2 times more phosphate and 1.8 times as much nitrate, compared to non-colonised roots [61]. This is associated with great increases in biomass and root proliferation : inoculation of *Eucalyptus globulus* with *Descolea maculata* increased total biomass by almost 70% and root length by 42% [68].

Arbuscular mycorrhizal fungi (AMF) form a different type of association with the roots. Root morphology is not affected to the same extant as those associated with ECM and diagnosis of infection necessitates laboratory procedures. Over 70% of plants form AM associations including many trees, shrubs, grasses, herbaceous perennials, pteridophytes and bryophytes. AMF are obligate biotrophs and have yet to be isolated and grown for any length of time in pure culture. They are characterised by non-septate mycelium and the formation of arbuscules (literally 'little trees') within the root cortex. These are one of the most fundamental AM structures, being the main site for the exchange of metabolites between host and fungi, and may only persist for a few days. Vesicles (balloon-like structures) which may have a storage or reproductive function are sometimes also produced. AM are formed with zygomycetes such as *Glomus, Sclerocystus, Acaulospora* and *Entrophospora* which form both vesicles and arbuscules, and *Gigaspora* and *Scutellospora* which form arbuscules only. Over 140 species of AMF are currently recognised [69], although there has been some debate over the concept of species in a group of fungi for which no sexual stage has yet been detected. Taxa are identified by spore and sporophyte morphology, and increasingly by

molecular techniques. Commercial inoculum is available, although not widely used by nurseries or landscape practitioners.

Most AMF infections are initiated by contact with a pre-existing mycelial network, or from propagules, such as infected plant roots, asexual spores, vesicles and organic matter colonised by AMF [45]. Propagules are dispersed by water, air currents, soil fauna, some larger animals and also by human activities. Infection occurs via root hairs or directly through the root epidermis by appressoria. Host roots are only infectable for a limited time, usually before any secondary thickening occurs, and plants may be infected concurrently by more than one species of AMF [45].

Plants vary in their dependency on mycorrhizas according to root system characteristics, habitat conditions and successional status [45, 61, 70]. Genetically determined root system characters, which reduce the ability of the plant to obtain soil resources, may predispose the plant towards dependency. Thus coarse-rooted species such as *Citrus* tend to be highly dependent; fibrous-rooted species such as wheat, less so. Recently a gene has been identified governing dependency in *Triticum* [71], suggesting a genetic basis. High levels of soil nutrient also tend to uncouple the symbiosis, as the plant still has to bear the cost of maintaining the fungal mycelium, which may consume up to 10% of total plant photosynthate [61]. However it should also be remembered that non-mycorrhizal nutrient uptake also requires energy. Graham and Eissenstat [72] demonstrated under field conditions of adequate P nutrition, that indigenous AMF reduced the growth of a range of citrus rootstocks by 5-17%, compared to rootstocks treated with benomyl to control AMF development. However, it should be noted that this treatment in itself would have controlled some soil pathogenic fungi. Plant successional status also determines mycorrhizal strategy: early successional plants with well-branched root systems,

adapted to high levels of disturbance or low nutrient levels tend to be either non-mycorrhizal ruderals, or else facultative mycotrophs, for example many grasses, shrubs and smaller trees [45, 70].

3.3. BENEFITS OF AMF.

3.3.1. Effects on growth and biomass production

The majority of benefits attributed to AMF have concentrated on increased biomass, improved nutrition and enhanced stress tolerance. A survey of the literature (summarised in Table 3.1) indicated increased biomass production (shoot height, shoot and root dry weight) across a range of woody species. Most of these trials were conducted on commercially important species such as apple, included in this review owing to their close taxonomic relationships with rowan and other native broad-leaved trees that become infected with AMF. Results such as these, which suggest increases of several magnitudes due to inoculation, would, if replicable in nursery and field conditions, have implications for plant quality and survival. However, a closer look at some of the experimental evidence reveals that few were carried out under non-sterile field conditions, and that results were modified by host-AMF interactions and also nursery practices such as nutrient status and choice of substrate.

Of the trials cited here, only that of Plenchette *et al.* [73] was conducted under non-sterile field conditions, where both inoculated and non-inoculated control plants became colonised by indigenous AMF. Despite this, inoculated plants still outperformed non-inoculated plants. Morin *et al.*[74] observed height increases of 200% following inoculation with a range of AMF species, although effects disappeared after 12 weeks. This phenomenon of early growth benefits was also recorded by Lovato *et al.* [75] on cherry microplants. Four weeks after inoculation stem height and diameter were greater

than controls, however by 13 weeks these effects had disappeared. The results of Douds *et al.* [76] and Kormanik *et al* [77] are particularly large, and may indicate other factors such as excessively low nutrients inhibiting the growth of the non-mycorrhizal control plants. For example, the control plants of Douds *et al* [76] grown under low nutrient levels had a shoot dry weight of 0.1 g compared to high nutrient controls which weighed 1.7 g.

Table 3.1. Experimental evidence for increased growth and biomass production in woody plants following inoculation with arbuscular mycorrhizal fungi.

Host	Trial conditions	Height[a]	Shoot dry weight [a]	Root dry weight [a]	Reference
Apple	Sterile soil mix	-	2.1	1.6	Mosse [78]
Apple	Non-sterile soil	2.4	3.6	2.4	Plenchette *et al.* [73]
Apple	Sterile soil mix	2.0	1.7	2.0	Morin *et al.*[74]
Acer negundo *Fraxinus pennsylvanica;* *Prunus serotina*	Sterile soil in amended field plots	3.5 5.6 5.5	30 58 73	40 66 74	Kormanik *et al.* [77]
F. pennsylvanica	Sterile soil mix	4.7	56	33	Douds *et al.*[76]
F. excelsior *Prunus avium*	Peat-based	2.3 ns [b]	- -	- -	Lovato *et al.*[75]

[a] Ratio of inoculated to non-inoculated plants for each parameter
[b] Not significant
[c] Data given for root fresh weight

Few studies have addressed the field performance of inoculated plants, where there may be competing indigenous AMF. Visser *et al.* [79] used soil containing AMF and *Frankia* propagules, as inocula for *Elaeagnus commuta* and *Sherpherdia canadensis* which were transplanted onto mine spoils. After one year there were increases in shoot height (X2), shoot dry weight (X5) and root dry weight (X4) in inoculated compared to non-inoculated plants. However higher mortality rates were experienced by the inoculated plants during the first winter, an unexpected finding which they suggested was due to insufficient hardening, and differences in physiological or nutritional conditions

between inoculated and non-inoculated plants. Morrison *et al* [80] observed that while inoculation during the nursery stage increased root colonisation by AMF, there were no differences in stem growth. When the trees were transplanted to a non-sterile field site, no growth effects after two years were observed in *Malus* or *Fraxinus*, although stem diameter was significantly increased in *Sorbus aucuparia*. This indicated that inoculation might in some cases improve transplant growth despite the lack of any benefits to early growth. Delisle [81] also transplanted one year old *Fraxinus pennsylvannica* inoculated with *Glomus intradices* onto a range of former clear-cut forest and abondoned agricultural field sites. After 4 years no differences were observed between inoculated and non-inoculated plants in height, root collar diameter or survival, although there were significant correlations with soil wetness.

3.3.2. Effects on plant nutrition

Traditionally, reviews of AMF have concentrated on improvements to plant nutrition and more efficient use of applied nutrients, particularly of relatively immobile ions such as phosphate. Improved nutrition would enable plants to tolerate sites with low nutrient status, as well as reduce some of the financial and environmental costs of fertiliser inputs at both the nursery stage and post-planting. Studies have indicated improved uptake of a range of plant nutrients including phosphate, magnesium, iron and copper [43, 74, 78]. Where several levels of nutrients were applied, mycorrhizal plants tended to maximise growth at lower levels than non-mycorrhizal plants. Koch *et al.*[82] used 7 levels of P ranging from 0 to 1000 ppm : mycorrhizal plants maximised height and dry weight at 100 ppm, non mycorrrhizal at 200 ppm. Above these levels, height and dry weight was decreased. Gardiner and Christianson [83] observed an interaction between AMF species and P level in pear: up to 250 ppm, growth was superior in plants inoculated with *G. intradices,* however above that level growth was superior in the

plants inoculated with *G. deserticola.* Plants inoculated with *G. deserticola* and the non-inoculated plants continued to grow in size as P levels increased, however at the maximum level of P in the trial (400 ppm) inoculated plants were still significantly taller (X 1.7) than the controls. Morrison *et al.*[80] recorded negative or non-significant responses to inoculation for a range of landscape trees and shrubs under nursery conditions of high fertility.

Johnson *et al.* [61] suggested that the external mycorrhizal mycelium can supply up to 80% of a plant's requirement for P and 25% for nitrogen. Mosse [84] cited evidence using labelled P-isotopes that mycorrhizal roots took up more P than non- mycorrhizal roots. Gianinazzi-Pearson & Gianinazzi [85] suggested that increased P uptake was a function of the size and spread of the external mycelium, the presence of specialised enzymes concerned with P uptake such as alkaline phosphatase, and more efficient transport and exchange processes. However they ruled out the possibility that mycorrhizal roots were able to mobilise P sources unavailable to non-mycorrhizal roots. Sieverding [86] noted that the external mycelium of AMF increased the volume of soil explored per cm of plant root by a factor of 12 to 15.

3.3.3. Effects on rooting structure

Studies have also indicated that AMF affect the size and structure of root systems, as well as root longevity. These effects would have implications for the quality of planting stock (root to shoot ratio), and the rapid establishment of an efficient root system able to survive the transplanting process. Hooker *et al.* [42] observed that inoculation increased root length in primary and tertiary roots by up to 100%, and root branching (the number of laterals per unit length of root) by up to 600%. Berta *et al.*[43] in *Prunus cerasifera* observed increased root branching in all orders of roots, the effect being most

pronounced in first order laterals where increases of 300% were recorded. This resulted in second and third order roots comprising a greater proportion of the root system in mycorrhizal plants, compared to non-mycorrhizal plants where the bulk of the root system comprised primary roots. Tisserant *et al.* [87] observed differences in root systems in mycorrhizal *Platanus acerifolia*, where the root system was dominated by third-order laterals, compared to non-mycorrhizal plants where second order roots were more numerous. Hooker *et al.* [44] also observed changes in root longevity in *Populus*: only 16% of roots colonised by AMF survived longer than 49 days, compared to 49% of non-colonised roots. This would appear to indicate that mycorrhizal root systems were younger and more metabolically active, and would have implications for carbon and other soil nutrient fluxes.

The above studies highlight the difficulty in separating effects of root system morphology due to improved nutrition (attributable to AMF) from direct fungus effects on root functioning. Hooker *et al* [42] observed that AMF-induced effects on root branching were in excess of those achievable in control plants supplied with additional nutrients. Berta *et al.* [43] supplied both mycorrhizal and non-mycorrhizal plants with excess P, and although this lead to higher internal P concentrations in mycorrhizal plants, they concluded that differences in P uptake was associated with differences in root system size. Tisserant *et al.*[87] observed that increased production of lateral roots coincided with increased fungal activity and enhanced P uptake. These studies, while linking effects on rooting structure with nutrient acquisition, also suggested the involvement of changes in root meristematic activity, root mitotic index and fungus produced auxins, cytokinins and gibberellins.

3.3.4. Effects on abiotic and biotic stress tolerance

As discussed in Section 2.2, urban landscape sites may be hostile to plants. Table 3.2 summarizes the main site stress factors, identified by Bradshaw *et al.* [12] which may be addressed by AMF, either directly by increased tolerance or indirectly through effects on rooting structure and nutrient uptake. It should be noted that tolerance of certain of these factors, such as waterlogging, soil conditions and toxicity, will also be effected by the ecological tolerances and adaptations of individual AMF taxa. These site stress factors will be used to structure the decision model described in Chapter 12.

Table 3.2. Site factors affecting tree growth on urban landscape sites which may be addressed by AMF.

Factor	Ranking*	AMF Effect	Mechanism
Drought	1	Direct Indirect	Tolerance Effects on size and structure of root system
Weed competition	2	Indirect	Influence on plant competition e.g. suppression of non-mycorrhizal taxa.
Soil structure, including compaction	4	Questionable	Positive effects on formation stable soil aggregates. May be inhibited by effects of compaction.
Waterlogging	4	Questionable	May be inhibited
Lack of nutrients and organic matter	5-6	Direct /indirect	Effects on rooting structure and physiology of nutrient uptake
Toxicity	6	Direct	Some species/ecotypes adapted to hostile local conditions
Road salt; air pollution	7-8	Questionable	May be inhibited
Pests and disease	8	Direct	May increase tolerance of some soil pests and diseases

[1] Score based on occurrence and severity. (Adapted from Bradshaw *et al.*, [12])

Drought

Drought is one of the most chronic causes of tree decline and may be a consequence of both plant and site factors. The effects of AMF on root system morphology and the implications for root regeneration and resource acquisition from the soil, have already reviewed (Section 3.3.5). There is also evidence that AMF have positive physiological effects on drought tolerance in the host plant. Gianinazzi-Pearson and Gianinazzi [85] suggested that drought tolerance was linked to phosphate status, rather than higher hydraulic conductance of mycorrhizal roots, as the addition of P to non-mycorrhizal roots eliminated differences in resistance to water transport. Lovato *et al.* [85] implicated the role of AMF in regulating stomatal movements, photosynthetic activity, proline accumulation, leaf elasticity and water retention in the symplast. Allen and Allen [89] observed that AMF may alleviate the effects of 'ecological crunches' such as drought. In a field trial involving the xeric grass *Agropyron smithii*, inoculation decreased stomatal conductance and increased leaf water potential during the driest part of the year, thus increasing water uptake. Zajicek *et al.* [90] also noted AMF effects on drought tolerance using the forb species *Liatris aspera* and *Baptista australis*. In a pot experiment with varying levels of drought stress and nutrient levels, plants infected by AMF were larger than non-inoculated plants at low nutrient levels, although the effect was less pronounced at higher nutrient levels. However, when the seedlings were transplanted to disturbed sites subjected to drought stress, mycorrhizal plants were significantly larger regardless of nutrient levels. They suggested that pre-inoculation of seedlings with AMF adapted to site conditions might be particularly advantageous in low maintenance landscapes.

Weed competition

Indirect effects of AMF on weed competition may be attributed to AMF. If AMF plants are larger and better able to obtain soil resources, weed competition may be of less importance. Many ruderal, weedy species also tend not to be mycorrhizal, for example Brassicaceae, Chenopodiaceae and Caryophyllaceae. These plants may possess different metabolic pathways for P absorption [91] , as well as secondary metabolites and root structures which enable the roots to resist colonisation by AMF [45]. Francis and Read [62] suggested the possibility of manipulating AMF as 'bio-herbicides' of non-mycorrhizal plants. Using 'split-pots' permeable to mycelium but not roots, they demonstrated that the growth and survival of non-mycorrhizal plants was adversely affected by the presence of a mycorrhizal host plant. Exposure to AMF increased survival of *Centaurium erythaea* from 20% to over 80%, while decreasing survival of *Arenaria hirsuta* from 85% to 20%.

Soil physical conditions

Miller and Jastraw [92] highlighted the importance of AMF hyphae in the formation of stable soil aggregates, a finding which has direct application to land restoration and soil conservation. Fungal hyphae and fine plant roots physically bind soil particles into micro- and macro-aggregates, which are then further cemented by mucilage and polysaccharides produced by plant roots. On soil compaction, Nadian *et al.* [93] observed that, although there were some differences between AMF species on shoot growth and P uptake below bulk densities of 1.6 Mg m^{-3}, above 1.75 Mg m^{-3} no mycorrhizal growth increases were observed, suggesting that soil compaction inhibited the growth of some taxa of AMF. This effect was attributable to the decreased oxygen content of the soil atmosphere, changes in pore size distribution, which even the smaller diameter of mycelium could not penetrate, and increased ethylene production.

Soil toxicity

Trappe [94] observed that mycorrhizal roots were better able to cope with the effects of high arsenate content in orchard soil, caused by excessive use of lead arsenate pesticides. Non-mycorrhizal roots tended to be stunted and unable to respond to applied nutrients, in contrast to mycorrhizal roots which also appeared to be healthier. Klironomos [95] suggested that AMF aided the survival of sugar maple (*Acer saccharum*) in low pH soils: sugar maple are very sensitive to low soil pH, and increased soil acidification caused by atmospheric pollution, has lead to their decline in S. E. Canada. Thus, there may be some potential in selecting AMF for tolerance of particular soil toxic conditions.

Pests and diseases

Reviews by Dehne [96]; Sylvia and Williams [97]; Fitter and Garbaye [98] and Linderman [99] have suggested that in the majority of cases AMF reduce the occurrence of pathogen damage. Possible mechanisms have included enhanced nutrition and hence reduction of abiotic stress, producing a healthier plant better able to withstand infection; competition with pathogens for infection sites and photosynthate; morphological changes to roots, and changes in the chemical constituents of roots and root exudates.

Utkhede *et al* [100] noted that apple replant disease, which is caused by actinomycetes which accumulate in soils where Rosaceae hosts, including rowan are grown, was markedly reduced by inoculation with *G. mosseae.* Newsham *et al.* [101] demonstrated that inoculation with *Glomus* sp. protected the annual grass *Vulpia ciliata*, from the root pathogen *Fusarium oxysporum* under field conditions. The grass was inoculated with factorial combinations of the pathogen and mycorrhiza before being planted out on the sites from which the inocula (pathogen and mycorrhiza) had been isolated. Effects of

AMF inoculation were insignificant when there was no disease present, but shoot biomass and root length were increased when the grass was exposed to both *Glomus* and *Fusarium*. These findings have implications for plant health in the nursery and on sites. Increased tolerance of pests and pathogens may allow some pesticides to be reduced, and also enhance plant fitness under field conditions. Pinochet *et al.* [102] inoculated micropropagated Myrobalan plum rootstocks with two species of AMF, prior to planting in soils infested with the nematode *Pratylenchus vulnus*. Although AMF inoculation had no effect on pest numbers, host tolerance to the nematodes was increased, by stimulating plant nutrition and growth.

3.4. ECOLOGY OF AMF

AMF occur in most terrestrial ecosystems, with the exception of some aquatic, saline, arctic and disturbed habitats [45, 103]. Read [104] suggested that for each ecosystem "... a predominant mycorrhizal type is recognised in which selection has favoured fungi with the ability to mobilise or capture the growth-limiting nutrient characteristic of that part of the gradient." Thus, according to Read's model, ericoid mycorrhizas characterise high altitude and latitude heathlands, where low pH, toxicity and nutrient sequestration limit plant growth; ectomycorrhizas dominate boreal and forest habitats where nitrogen is limiting, and arbuscular mycorrhizas dominate less-extreme mixed-species habitats where phosphate tends to be the limiting factor. This over-simplification has been criticised by Brundrett [45] and Klironomos [95] as there is some cross over in the occurrence of mycorrhizal types, although the functioning of each type of symbiosis may not be optimal in all habitats.

Despite their near-ubiquitous distribution, AMF are not homogenous organisms, but demonstrate a range of environmental tolerances and adaptations to factors such as soil

type, pH, nutrient status, salinity, moisture, temperature, disturbance and the presence of other soil biota, including indigenous AMF [45, 97, 105]. These factors influence the occurrence, competitiveness and functioning of AMF in different natural and managed ecosystems, and may be exploited in inoculum selection programmes.

Soil pH has a significant effect on AMF distribution. Sieverding [86] observed that *G. mosseae* had only ever been observed in soils with a pH greater than 5.5, whereas *Enterophospora columbiana* was found in soils with a pH less than 5.5. In a field study, Porter *et al.* [106] noted the occurrence of *Acaulospora laevis* in soils with pH less than 6.4, and *Glomus* WUM3 in soils above pH 6.8. When isolates of these species were grown in soil differing from their site of origin +106, they recorded lower root infection, spore production and germination. However these effects were largely overcome by adjusting soil pH. Differences in AMF functioning, attributable to pH, were also demonstrated by Abbott and Robson [108]. In a pot experiment using subterranean clover, *G. fasciculatum* was able to infect host roots and improve plant growth across a range of pH from 5.3 to 7.5. However, *Glomus* WUM16 was only able to infect roots at pH 7.5; below pH 5.3 the fungal hyphae were unable to extend beyond the root into the soil matrix.

Several studies have also suggested the occurrence of intra-specific variation in AMF. Haas and Krikum [109] noted that there was much variation in the ability of isolates of *Glomus macrocarpum* to colonise roots and affect growth in bell pepper. Isolates varied not only between site of origin, but also within a single soil sample. Bethlenvalvay *et al.* [110] studied host response to morphologically similar isolates of *G. mosseae* collected from arid, semi-arid and mesic areas, when grown under standard conditions. Despite similar internal nutrient concentrations, the isolates varied in their effects on growth and

leaf conductance. These findings lead them to propose the term 'edaphotype' to refer to "...intra-specific variants of soil fungi that are of different edaphic origin and elicit distinct physiological responses from plants when grown under uniform conditions." Stahl and Christensen [111] examined the environmental tolerances of geographically distinct populations of *G. mosseae*. No information was given on the native habitats of the isolates, although small differences in fungal morphology were observed. Under experimental conditions, the three populations were found to vary significantly in their tolerance of soil type, soil moisture and soil temperature. It was suggested that both phenotypic plasticity and genotypic adaptation were involved in this phenomenon.

Population dynamics are also affected by changes in site conditions that favour particular species and isolates [112]. It is therefore important to appreciate the effects of disturbance and changing soil and vegetation management regimes on species composition, host infectivity, effectiveness (ability to promote host plant growth) and persistence in the environment.

The species diversity of AMF populations tends to decline under high levels of soil disturbance: more than 20 species have been recorded in some natural ecosystems, compared to fewer than 7 in disturbed habitats (Table 3.3). Sheppard *et al.* [113] observed in Kenya that *Acaulospora morrowiae, A. melea* and *A. scrobicularia* were abundant in a broad range of soil types; while *Scutellospora* spp. occurred exclusively in disturbed agricultural soils, and *Glomus* only occurred in undisturbed forest soils. Sieverding [86] noted similarly broad environmental tolerances in *Acaulospora* spp. and *S. pellucida* and indicated that there was a need to identify fungal species that were tolerant of disturbance. Site disturbance, including excessive soil cultivation may damage the

mycelial network and disrupt the spatial distribution of propagules and the organisms that act as propagule vectors

Table 3. 3. Number of AMF associated with particular site conditions

Author	Habitat	No. species	AMF present
Walker *et al.*[114]	Poplar plantation (Iowa)	10-12	*Glomus* spp. *Gigaspora* spp. *Acaulospora* spp.
Vosatka [115]	Mine spoils, north Bohemia	1-7	*Acaulospora* spp. *Gigaspora* *spp. *Glomus* spp.
Sieverding [86]	Natural ecosystem Low input agriculture High input agriculture (Tropics)	16-21 10-15 6-9	Not indicated
Sheppard *et al.* [113]	Farm soils, Kenya	6-10	*Acaulospora* spp. *Scutellospora* spp. *Glomus spp.*
Zajicek *et al.*, [90]	Road cut soil Mine spoil New housing scheme	5 4 6	*Glomus* spp. *Sclerocystis* spp.

* Usually restricted to the New World (Walker, pers. comm.)

The species composition of AMF populations following disturbance is governed by several factors. These include the initial species diversity, type of habitat, presence of suitable host plants, and also the time of year when disturbance occurs. Jasper *et al.*[116] noted that certain habitats contain more robust AMF propagules (spores and mycelium): soil from a productive pasture was found to contain 10-25 times the number of infective propagules compared to a heath or forest soil. Following soil disturbance, soil infectivity (ability to cause infections in host plants) was unaffected in the pasture soils but reduced by 50% in forest and heath soils. This was attributed to the ability of some AMF hyphae to withstand damage, as well as the higher proportion of mycorrhizal host plants growing in the pasture soil. Miller and McGonigle [117] observed that the extra-radical mycelium of some species can remain viable between seasons and was therefore capable of re-establishing infections when conditions were more favourable to root

growth. Pattinson and McGee [118] demonstrated that periodic wetting and re-drying in fallow cotton fields can be more damaging to soil mycelium than disturbance.

Sutton and Barrow [119] observed that spore populations in Ontario varied seasonally, increasing in late summer and autumn and then declining until the following summer, which they attributed to host growth stage and climatic conditions. Walker *et al.*[114] recorded both spatial and seasonal variations in spore population. Spore clusters occurred in relation to root location and native vegetation, and population levels tended to follow changes in soil moisture levels and root phenology. These findings indicate that timing of disturbance may also affect the species composition: species sporulating prior to disturbance are more likely to persist.

3.5. MANAGEMENT OF AMF

The ubiquitous nature, both in terms of host range and habitat distribution, have lead many to consider management of the symbiosis unnecessary [120]. However, where plant growth is likely to be affected by factors which may be overcome by AMF, or where indigenous populations are low, unevenly distributed or ineffective, it may be advantageous to consider inoculation or manipulation of indigenous AMF populations [86, 121].

3.5.1. Situations in which AMF management may be necessary

Plant growth on landscape sites may be constrained by hostile climatic and soil factors, some of which may be addressed by AMF (Section 3.3). However, many nursery and land management practices eliminate or selectively modify AMF populations. On nurseries, disinfection practices such as soil sterilisation, use of inert media and biocides, as well as high fertiliser regimes reduce AMF. Danielson and Visser +122

surveyed a sample of trees and shrubs from 7 Canadian nurseries that supplied plants for land reclamation schemes. Few one-year old plants were mycorrhizal, although if plants had been over-wintered outdoors, an erratic level of infection from fine endophytes was detected. Morrison *et al.*[80] also sampled a range of nursery grown landscape trees for AMF colonisation including *Sorbus aucuparia*, and recorded levels of less than 2% in the majority of cases. Despite this, few nurseries consider inoculation, perhaps believing that plants will become mycorrhizal anyway when planted out on sites. However many landscape sites also lack sufficient or effective AMF populations, particularly following site construction processes and topsoil storage [92], or if the site has been derelict or recently reclaimed. The native vegetation cover which supported the symbiosis, will have been removed; soils compacted by the use of heavy machinery and inoculum-free subsoil exposed [70]. Disturbance has a direct effect on reducing or qualitatively modifying AMF populations (Section 3.4). The factors limiting plant growth and AMF are thus inextricably linked, and hostile landscape sites may be both a cause and consequence of reduced AMF populations.

Land management practices will also selectively reduce or modify the indigenous population, so that it is composed of species and isolates which are ineffective symbionts, provisioning their own growth rather than that of the host [123]. These practices include intensive soil cultivations, excessive use of fertilisers and pesticides, continuous mono-cropping or growth of non-mycorrhizal or low AMF-dependent crop cultivars. Where there has been a change in land use or an 'exotic' plant species is to be planted on the site, the indigenous AMF species may not be the most appropriate fungal partners.

The AMF status of a site may need to be assessed both quantitatively and qualitatively, by determining the site's Mycorrhizal Inoculum Potential (MIP) or by bio-assay. The critical population level (number of AMF propagules necessary to benefit plant growth) has only been determined for a limited number of horticulturally important crops [86] and not at all for important landscape genera which may depend on the symbiosis. It is recommended that indigenous AMF populations are qualitatively assessed for effectiveness (ability to benefit plant growth) by bio-assays, where inoculated plants are grown under controlled conditions. Sieverding [80] recommended that at least two-thirds of the fungal species present should be "highly effective" at improving plant performance.

3.5.2. *Enhancing indigenous AMF populations*

If investigations suggest that the indigenous AMF population is below a critical level or ineffective, then steps may be taken to either enhance that population by site management practices [124] or to consider inoculation of planting materials or even whole sites. As has been suggested (Section 3.4) AMF species and isolates may be highly adapted to particular edaphic conditions and there is some evidence of preferential host-endophyte associations. If land practices favour certain AMF, then these practices may be exploited or modified to enhance the desired species composition. Exact information of optimal management practices for particular AMF species, and the ideal host-AMF combination for each situation, is at best patchy, and so it may be worthwhile adopting practices which favour diversity, thus increasing the probability that at least some of the population will be beneficial.

There is an adequate body of information on modified agronomic practices that may be applicable with imagination to landscape management practices (Table 3.4).

Table 3.4. Management practices to encourage indigenous mycorrhizal fungi.
(Adapted from Sieverding [86]; Hitchmough, [125]; Hooker and Black, [126] Azcon-Aguilar and Barea, [127]; Smith and Read [128])

Management Practice	Consequences	Landscape Application
Land preparation	Minimise soil cultivations to avoid damage to mycelial network	Cultivations may be unavoidable if necessary to provide workable substrate, especially if compacted. Avoid cultivations once planted
Cropping sequence	Avoid soil fallows or bare soil. Avoid monocultures Crop rotations and inter-cropping with AMF-dependent spp.	Avoid bare ground if site not to be planted immediately and sow mycorrhizal 'bait' plants. Plant diverse species communities.
Crop selection	Select for AMF dependency and responsiveness : root system characteristics and root exudates.	Incorporate into plant selection procedure?
Fertilisers	Slow-release (e.g. rock phosphate) or reduced applications Soil amendments, especially plant residues and manure.	Amend management specification
Irrigation	Minimise: excessive irrigation may cause AMF propagules to be washed deep into soil profile, or else lost to soil run-off. May also reduce host-dependency on AMF	Amend management specification
Weed control	Weeds tend to be non-mycorrhizal therefore reduce AMF populations. Some weedy taxa may be antagonised by AMF. AMF may increase uptake of certain herbicides by host plant.	Amend management specification: once AMF network established in soil, weed control may be unnecessary.
Pest and pathogen management	Select for compatibility with AMF. Reduced applications	Amend management specification

Obvious differences exist between agronomic situations and landscape sites, particularly regarding severity of site hostility, range of planting materials, longevity of planting schemes and level of management skills. In general less intensive land preparation and management practices are more favourable to AMF, although AMF species may vary in their tolerance of agronomic practices. Compromises will need to be made between the level of management intervention to maximise host plant growth, while reducing levels of inputs to maintain its dependency on the association. Many of the practices to encourage AMF are equally applicable to the management of AMF-inoculated plants.

3.5.3. *Inoculation strategy.*

As with any landscape plant selection strategy aimed at matching plants to site conditions, AMF inoculants need to be selected with care to maximise site adaptations, compatibility with host plant and cultural regime, as well as potential to improve plant performance. Because of the heterogeneous and dynamic nature of sites, it may be advantageous to inoculate with a cocktail of species rather than a single endophytes. These selection criteria are summarised below in Table 3.5.

Plants may be inoculated at any production stage from seedling, cutting or microplant, through to final planting stock. However, it may be more cost-effective and economic to inoculate at an early stage, to reduce the quantity and hence cost of inoculation, as well as maximise early growth benefits. Decisions will need to be made about the purpose of inoculation: whether it is to affect plant growth in the nursery or final growing position. AMF isolates adapted to nursery conditions may not be optimal for the intended site, assuming that this is known at the production stage. Where contract

growing of planting stock is practised, there may be some potential to more accurately match inoculum characteristics to site conditions.

Table 3.5. Selection parameters for AMF inoculants
(Adapted from Sieverding [86]; Dodd and Thomson [121]; Mason and Wilson[129]; Janos[129]).

Parameter	Consequence
1. Culturability	Tolerant of inoculum isolation, production and storage practices
2. Edaphic adaptations (nutrients, pH, moisture, salinity, toxicity, soil or other substrate type).	Tolerant of nursery practices, growing media etc. Adapted to final site conditions
3. Specificity	Broad host range
4. Infectability	Ability to infect and colonise root systems
5. Effectiveness	Ability to increase nutrient uptake, protecting against root pathogens, stress-tolerance etc.
6. Robust propagules	Persistence and spread on site. Able to survive host dormancy
7. Competitiveness	Ability to compete with indigenous AMF and other soil micro-organisms

It has been suggested that indigenous AMF species may be better adapted to site conditions [128]. However where sites have been highly disturbed, and indigenous populations are depauperate, this may not be appropriate. Zajicek *et al.*[90] observed that on disturbed sites, introduced species may be better adapted than indigenous populations. Clark and Mosse [130] suggested that in conditions of extreme phosphate deficiency, any endophyte species would increase plant growth, however species selection becomes more critical under more fertile conditions.

Several researchers have observed host preferences for particular endophyte species. Kormanik *et al.*[77] observed that seedlings inoculated with *Glomus fasiculatus* outperformed those inoculated with a mixture of *G. mosseae* and *G. etunicatum*. Although there were differences between the number of propagules applied: 6600 spores of *G. fasciculus* per metre plot, compared to 850 of the *G. mosseae* / *G. etunicatum* mixture, they attributed differences to the erratic performance of *G.*

etunicatum in high temperatures and high soil phosphate levels. There were differences in the relative proportions of arbuscules, vesicles and hyphae produced by the various host-AMF combinations, which may have affected the functioning of the symbiosis. They also recorded differences in host responsiveness to AMF : *Prunus* was the most responsive, *Juglans* least so.

Gardiner and Christenson [183] noted that *Glomus deserticola* increased shoot dry weight in pear seedlings by X 1.7 compared to only X1.2 with *G. intradices* There were also significant differences in rootstock effects: M26 responded better to inoculation than either OTT3, P16 or P22. Morin *et al.*[74] noted that *Glomus versiforme* was the most effective AMF species in promoting growth in apple, but the slowest to perform, which they attributed to initial carbon drain in establishing an extensive mycelial network. Lovato *et al.*[75] found that inoculation with *Gigaspora rosea* but not *G. intradices* or *G. desserticola* on ash initially reduced height in comparison to non-inoculated controls, although by week 13 this effect was no longer significant. Pinochet *et al.* [102] demonstrated differences between endophytes in conferring tolerance of soil nematodes in plum rootstocks: *G. intradices* was more effective than *G. mosseae.* Other researchers who noted differences in host-endophyte preferences include Hooker *et al.* [42] in poplar and Berta *et al.* [43] in *Prunus*.

Commercial inoculum production has been hindered by the obligate symbiont nature of the fungus, as well as high production costs and issues concerned with quality control [128]. In addition, the inconsistent effects observed in many agricultural and horticultural conditions, have failed to persuade customers to adopt the technology [131]. The ideal inoculum formulation should be economic to produce and use, significantly

affect plant physiology, be free from contaminants, easily handled and have an adequate shelf life [123].

Inoculum formulations range from low-tech and low-cost soil-based methods, to production on inert materials, to surface-sterilised propagules, which have differing advantages and applications (Table 3.6).

Table 3.6. Comparisons and potential uses of inoculum formulations. (Adapted from Azcon-Aguilar and Barea [127]; Smith and Read [128]; Lovato *et al.*[95].

Method	Advantages/Disadvantages	Applications
Soil-based	Cheap to produce. Production requires few specialised skills. Needs soil sterilisation Bulky to store/transport. Unpredictable composition Risk of contamination. Storage life: months/years.	Production in isolated areas, low-input growing systems. May be applied at transplanting stage. Landscaping and forestry
Inert media (e.g. expanded clay, perlite, vermiculite etc.)	Intermediate cost. Intermediate skills: more careful control of watering and irrigation. More predictable composition. Less receptive to pathogens. Less bulky to store or transport. May be dried for extended storage.	Seedling inoculation. Forestry and landscaping
Surface sterilised propagules	Relatively expensive. Limited storage (controlled conditions) Highly skilled. Predictable composition. Free from micro-organisms	Research Micropropagation Hydro-seeding

For landscape purposes either soil-based or commercial formulations on inert media would be most appropriate. Soil-based formulations have been economically produced with relatively few inputs on nurseries and landscape sites, following protocols described by Gianinazzi *et al.*[132]; Sieverding [86]; Wilson *et al.* [120] and Kemery and Dana [133]. Results are less predictable, but may have a broader spectrum of effects. Inert formulations, being more expensive are appropriate for very high-cost specimen

plants, or else for substrate incorporation at an early propagation stage. Species composition is more accurately guaranteed, particularly if a 'super-endophyte' selection had been produced.

3.5.4. Managing inoculated plants

Many of the practices designed to enhance indigenous populations (Section 3.5.2) are applicable to the management of inoculated plants. A commitment is needed on behalf of nurseries and landscape practitioners is required to ensure the functioning of the symbiosis [134]. Where cultural practices have not been amended, particularly with regard to fertiliser regimes, irrigation and choice of potting media, the results have not always been beneficial and this may in part explain some of the reluctance to adopt mycorrhizal technology

Choice of growing substrate, particularly with regard to pH, cation exchange capacity, phosphate fixation, and physical properties such as air spaces and water-holding capacity, can affect the performance of many AMF [135]. Estaun *et al.* [136] demonstrated AMF preferences for two commercial peat-based potting mixes using micropropagated *Prunus* rootstocks. In one mix, shoot height and percentage infection were greatest with *G. intradices*; in the second mix, *Acaulospora laevis* increased shoot height, despite lower levels of root infection. However, when Lovato *et al.* [75] compared the effects of two substrates which varied in peat content: increasing the peat content for *Prunus avium* had a more significant effect on growth than either endophyte species or fertiliser level.

Johnson and Pfleger [137] and Lovato *et al.* [88] reviewed the effects of soil fumigants, herbicides and pesticides. In general, AMF are very sensitive to soil fumigants, but

that the effects of fungicides tended to depend on the formulation used, dosage and timing of application. Fungicides from the dicarboximide group (e.g. 'Captan') stimulated root colonisation and spore production at half the recommended rate, but reduced colonisation when applied at the higher rate. Fungicides from the group of substituted aromatic hydrocarbons, which include chlorothlonil, PCNB and the benzimadioles, consistently inhibited AMF. In contrast, two anti-oomycete formulations (fosetyl-Al and metalaxyl) were found to stimulate mycorrhizal colonisation. This was attributed to either an indirect effect of the fungicide on the host plant allocation of soluble sugars to roots exudates, or inhibition of micro-organisms antagonistic to AMF. Herbicides had few deleterious effects at low concentration, although Hamel *et al.* [138] observed that in the case of mycorrhizal apple plants, uptake of simazine, paraquat and dichlobenil was enhanced leading to host, but not AMF toxicity problems. Most insecticides had either no or only a slightly antagonistic effect, and nematicides were generally beneficial, as they tended to reduce populations of the nematodes which predate AMF.

3.5. SUMMARY

AMF can potentially improve the quality and growth of nursery plants and their subsequent survival and performance on landscape sites. However, few experiments have been conducted under realistic nursery or field conditions, where results may be modified by competing indigenous fungi, as well as management practices such as fertiliser and irrigation, biocide usage, soil cultivations and choice of growing substrate. There is also evidence that plant taxa differ in their dependency on the association, and that individual species and isolates of AMF demonstrate host preferences, and vary in their tolerance of cultural conditions, although exact requirements have not been specified. At present inoculation is "...more an art than a

science" [86]. The issues of host plant dependency on AMF, the response of AMF to cultural practices, selection of suitable AMF isolates, and their performance and persistence under field conditions, will need to be addressed if the use of AMF in landscape plants is to become a workable technology. Some of these issues will be explored in the experimental chapters of this thesis.

CHAPTER FOUR
ROWAN

4.1. INTRODUCTION

Rowan (*Sorbus aucuparia* L.) is widely used in landscape and woodland planting schemes to enhance diversity, conservation and amenity interests. It is a focus for folklore in Celtic countries, and its presence helps to define local landscape character. It is known to form arbuscular mycorrhizal associations and there is some evidence of colonization by ecto-mycorrhizal fungi [66]. Rowan was chosen for the present study because of its significance, and also because it has received little attention from other researchers, particularly with regard to provenance variations and mycorrhizal associations. This is surprising bearing in mind its success in a range of stressful habitats, an ability that may be explored as a model for plant survival in the hostile conditions characteristic of many urban landscape sites.

4.2. BOTANY

Rowan is a slender, smooth-barked tree, which reaches up to 20 m in height, although individual specimens up to 28 m have been recorded in the British Isles [139]. Roots are tough and fibrous. Gillham [140] recorded a mean root length of over 50 cm in one-year-old seedlings. Dutton and Bradshaw [17] observed a high tolerance of root desiccation: 68% of seedlings survived following 7 days' root exposure compared to only 8% survival in *Betula pubescens*. McEvoy and McKay [141] recorded root frost hardiness in two-year-old trees to $-5^{\circ}C$ and noted that rowan displayed very little seasonal variation in sensitivity to frost from the end of October to early March.

Stem girth is up to 75 cm; bark thickness to 0.5 cm, smooth and silver grey [142]. The tree habit is monocormic or polycormic, particularly if subjected to grazing pressure or

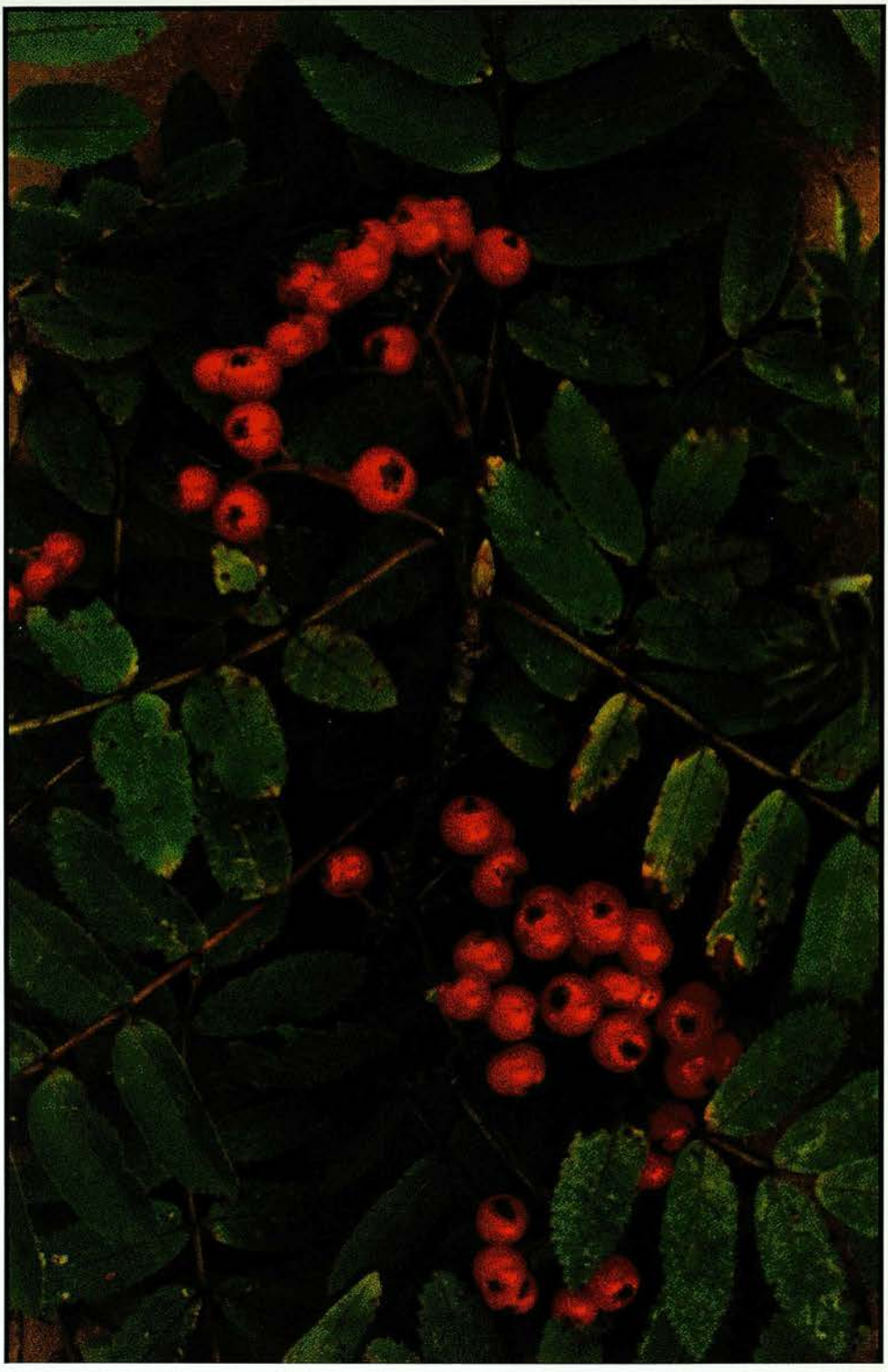
other stem damage. Branch angles are acute to stem, forming a narrow crown; branching pattern monopodial [143]. The timber is strong and fine-grained, with yellow sapwood and purple heartwood [144]. Buds are conical, black-ish in colour, with a few white hairs. The compound leaves are pinnate and hypostomatous, to 20 cm long, with 5-7 opposite pairs of coarsely-toothed leaflets, each up to 5 cm long, glabrous above, pubescent below when young. The inflorescence is a corymb of creamy-white flowers, which appear from late May to late June and have a fetid smell. Each flower is 6-10 mm in diameter, with 5 triangular sepals, 5 white petals, 3-4 styles and numerous stamens. The fruits are red or more rarely yellow, with a tough shiny skin and mealy flesh, to 1 cm diameter, ripening in Mid-September. (Figure 4.1). The seeds are relatively small, weighing 2-3 mg, and contain little endosperm: most of the seed food reserves are stored in the cotyledons [145].

The Latin binomial originates from *sorbum* meaning 'Sorb Apple' and *aucuparium*, implying 'bird-catching' from an ancient belief that 'the berries intoxicated the birds, rendering them more easily caught' [146].

4.3. THE USE OF ROWAN IN THE LANDSCAPE

Sorbus aucuparia L. is known vernacularly as Rowan (derived from Gaelic *ruadh-an* 'red-one' and Norse *rön* meaning 'rune', [144] and also Mountain Ash, from the similarity of the pinnate-leaves to those of the common ash (*Fraxinus excelsior)* and its abundance in hilly areas. In Gaelic it is referred to as *Caorthain* or *Caorunn* (Irish = *Caorthann*; Welsh = *Cerradin*), *Keirn* or *Cuirn*, whose derrivatives appear in many place names. Other names include Rodden, Quicken and Witchen, the latter being a reference to its alleged anti-witch properties.

Figure 4.1 Leaflet and fruit in Rowan



Rowan is a useful and attractive landscape tree, which is widely planted on a range of landscape sites, including upland shelter-belts, land rehabilitation schemes, as a component of open woodland, in nature conservation schemes, and as a specimen amenity tree for street planting, municipal parks and domestic gardens. It is not widely used in commercial woodlands. It has attractive flowers, good autumn colour and prolific orange-red berries which are highly nutritious to a range of birds and small mammals. Rowan tends to be light-demanding and shallow-rooted, preferring light-textured brown earth or fertile peat soils; they do not tolerate water-logging and are short-lived on calcareous soils above pH 7. They are extremely hardy, withstanding drought and wind, poor soils, exposure and atmospheric pollution, and are found at altitudes of over 900 m, higher than any other tree species in Britain [147, 148]. Korshunov [149] observed that it had ..."complete resistance to the severest of winter conditions in Eastern Europe".

Although no improvement programmes have been undertaken for forestry purposes, several amenity cultivars have been selected on the basis of morphology, such as 'Asplenifolia' which has fern-like foliage; 'Fructo-Luteo' which has amber-yellow fruits, and 'Sheerwater Seedling', an upright form used as a street tree [159].

In the past, its wood was esteemed by wood-turners for its compact grain, and was used for veneers, furniture, pulping, barrel hoops and archers' bows, as well as small domestic or agricultural implements. In parts of Eastern Europe and the former Soviet Union, it is grown as a minor fruit crop, where it is referred to as the "Northern Grape". It is used in pharmaceutical preparations; preserves and confectionery, and fermented to produce a drink resembling perry [151]. The fruits are particularly rich in vitamin C (up to 20 mg/100 mg fruit), and contain tannins, sorbitol (a sugar acceptable

to diabetics), sorbic acid, sucrose, malic acid, sorbin, oil and hydrocyanic acid. Because of the latter, they should not be consumed in excess. In general they are considered to have astringent and anti-diarrhoeic properties [152]. Varieties with large, and only mildly astringent fruits such as the cultivar 'Edulis' (synonymous with 'Moravica' and 'Dulcis') are grown for commercial fruit production; some breeding and selection programmes have been undertaken to improve fruit quality and pharmaceutical properties.

Rowan has also been used as a rootstock for many of the pinnate-leaved decorative sorbi (McAllister pers.comm.) and has been used successfully with a range of other rosaceaeous species including *Amelanchier, Cotoneaster* , *Pyrus,* Cratagus and *Chaenomeles* [153, 154]. Its use as a rootstock is particularly prevalent on Eastern European nurseries because of its ability to tolerate severe freezing conditions.

Traditionally, rowan was planted beside cairns, stone circles, homesteads, mines and well-heads, to protect against witches and evil spirits. According to Irish tradition, the first sacred berries were dropped by the Tuatha Dae Dannan, the peoples of the Goddess Danu, who latter became the Celtic deities [155] . It was believed to be the sacred tree of Bride, the pagan 'Great Goddess' and red, the colour of its berries, was considered to have particular powers against malevolent forces [156]. The wood, however was considered to be the most potent part of the tree, being was used to make divining rods or hung above stables and byres [157]. The felling of rowan trees was believed to bring ill-fortune. The fate of the Orkney Isles were thought to be bound up in a single tree: if so much as a single leaf were removed, the Isles would pass into foreign hands. A recent report in 'The Scotsman' cited the 'kissing tree' of Strachur,

a 70- year old rowan, which was in the path of road improvements. Fearing bad luck, the civil engineers arranged for it to be transplanted to the banks of Loch Fyne.

4.4. TAXONOMY.

The genus *Sorbus* L. is a member of the large and horticulturally important family of Rosaceae, which comprises 5 sub-families : *Spiraeoideae*, *Rosoideae*, *Neuradoideae*, *Prunoideae* and *Maloideae* [158]. *Sorbus* along with other genera such as *Amelanchier*, *Cotoneaster, Malus*, *Pyracantha* and *Pyrus* , belongs to the subfamily *Maloideae*, which is characterised by a concave receptacle that, together with the calyx, enlarges to enclose the fruits as a pome.

The genus *Sorbus* is considered to be one of the most taxonomically complex of the Rosaceae because of the considerable variation that exists within the vegetative and reproductive characters and the occurrence of introgressive hybridisation with subsequent apomixis and polyploidy [159, 160, 161]. Hedland [162] considered the genus to be in a process of active speciation and recognised 55 species; currently around 100 species are recognised [165].

Leaf form within the genus ranges from simple to lobed and pinnate, and has been used as the basis of most classification schemes. It has been suggested [159] that leaf form evolved in response to ecological conditions, vegetation phase, snow-fall and shoot freezing. Sorbus is representative of an ancient, warm-temperate mesophytic flora. According to Gabrielian [159] the primitive sections of the genus are concentrated in Eastern Asia, with the Caucasus and Balkans as secondary centres. However, Challice and Kovanda [163] considered that the European species exhibited the widest range of variation. The genus is classified into 6 sections, which are sometimes treated

as separate genera. Section Aucuparia contains the pinnate-leaved species such as Rowan, and is sub-divided into the orange-red fruited types which occur throughout the moister, cool-temperate northern regions, and the white-crimson forms which occur only in North and West China and the Himalayas.

Rowan is a fully sexual, diploid species (2n=34). Hybridisation to produce apomictic species has been reported with *S. arranensis* Hedl.; *S. intermedia* Erhl. (Purs.); *S. aria* (L.)Gaertn. and *S. rupicola* (Syme) Hedl. which further confuse the taxonomy of the genus in the British Isles [160, 161, 164].

Five subspecies of *S. aucuparia* are distinguished in Europe [165]:

1. ssp *aucuparia* which occurs throughout most of the range of the species, but tends to be rarer in the south
2. ssp *glabrata* (Wimmer &Grab.) Cajender, which occurs in northern Europe and the mountains of central Europe
3. ssp *fenenskiana* Georgiev & Stoj, which only occurs in Bulgaria
4. ssp *praemorsa* (Guss) Nyman, which occurs in Southern Italy, Sicily and Corsica
5. ssp *sibiria* (Hedl.) Krylov, which only occurs in NE Russia.

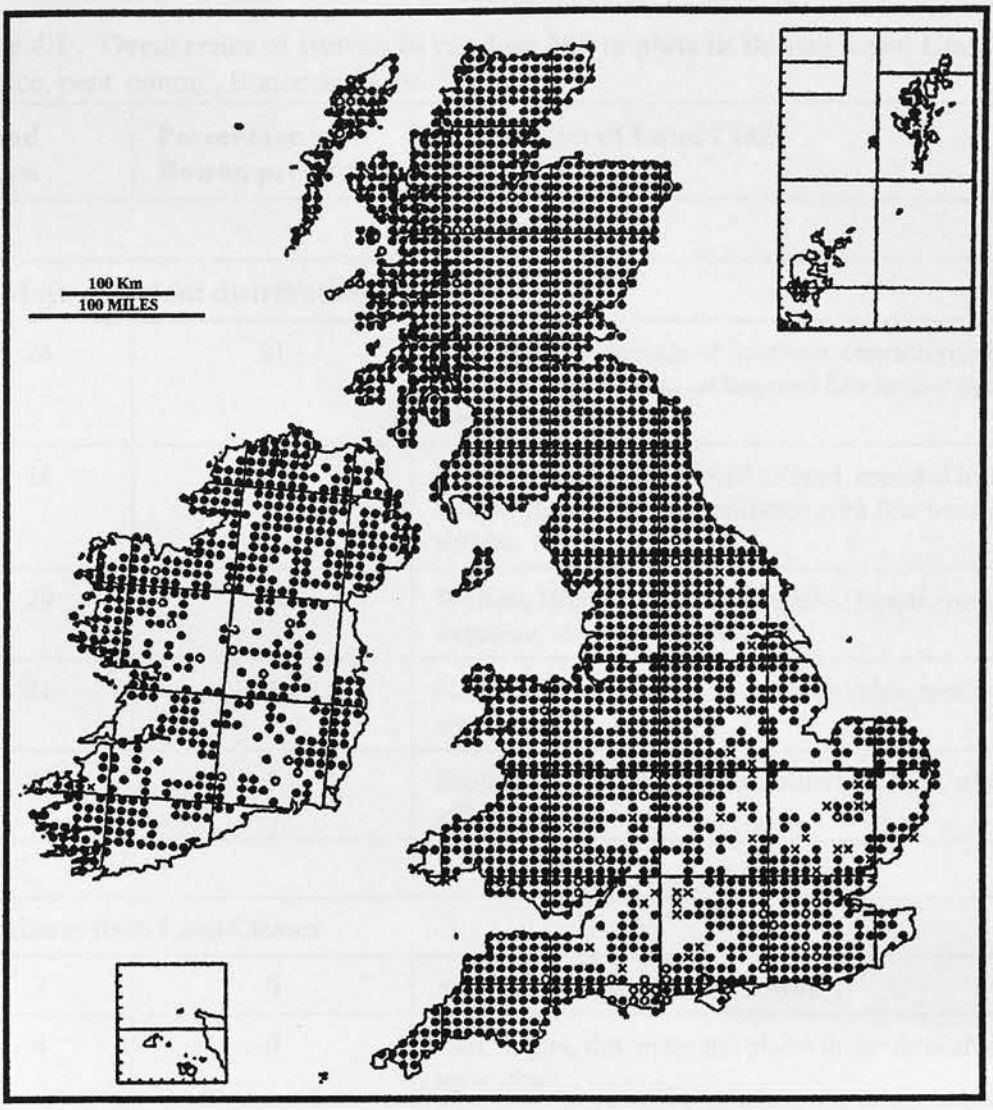
4.5. ECOLOGY AND RESPONSE TO ENVIRONMENTAL FACTORS

Rowan is a stress-tolerant competitor (S-C), "...adapted to relatively undisturbed conditions experiencing moderate intensities of stress'. This strategy is typical of most woody plants that occur in unproductive habitats, or the later successional stages of more fertile terrain [39,166].

Distribution

Sorbus aucuparia is widely distributed throughout most of Europe, north Asia minor and the high mountains of Morocco [165]. It is widespread throughout the British Isles, with the exception of central eastern England and central Ireland, as indicated in Figure 4.2, which is taken from the Atlas of the British Flora [167]. A survey made of rowan in 1990 by the Institute of Terrestrial Ecology (ITE) related the abundance of rowan in random 200 m^2 plots to Land Classes (Table 4.1). The system of Land Classes was devised for use in landscape planning, and integrates map-readable information relating to geology, topography, climatography and human artifacts [168]. The 1990 data indicate that rowan occurs predominantly in Land Classes 26, 18, 29, 21, and 22 which relate mostly to the mountain and coastal fringes of North-west Scotland. It is entirely absent from Land Class 23 includes high mountain summits, suggesting an altitudinal cut-off; the alluvial clays of the Midlands plains (12, 13); lowland coastal areas (8,14); limestone (2) and the extremely exposed habitats of far North-west Scotland (31, 32) where few trees occur.

Sorbus aucuparia is mainly restricted to wooded sites, particularly on non-calcareous strata, but has also been recorded from skeletal habitats including rocky outcrops, lead mines and wastelands [166]. In the uplands of Scotland, rowan is associated with the native Caledonian Pinewoods, and is occasionally found in pure stands, particularly in the west Highlands, where it may have replaced oak [169]. Edaphic requirements are similar to those of birch, in that it is distinctly favoured by acidic, non-waterlogged conditions, although it can persist at higher altitudes and is more shade tolerant than birch.



● 1950 onwards (GB – 2090, Ir. – 522, Ch. Is – 1)
○ before 1950 (GB – 53, Ir. – 52, Ch. Is – 0)
× introduction (GB – 46, Ir. – 2, Ch. Is – 0)

Figure 4.2. Distribution of Rowan in the British Isles.
(From: Flora of the British Isles [167])

Table 4.1 . Occurrence of Rowan in random 200 m plots in British Land Classes.
(Bunce, pers. comm., Bunce and Last [168]).

Land Class	Percentage with Rowan present	Description of Land Class
1. Most abundant distribution in Land Classes		
24	21	North-west Highlands of Scotland, characterised by badly-drained slopes, widespread forests and open range lands.
18	12	South-western Uplands of Scotland, rounded hills, some steeper slopes; varied moorlands with few trees or shrubs.
29	11	Western Highlands, rugged variable terrain, rock exposure, streams and lochs.
21	8	North-east Highlands, steep stream-sides, rocky outcrops with varied vegetation.
22	7	South-east Uplands, rounded moorland hills, often afforested.
2. Absent from Land Classes		
2	0	South-east England, chalk downs.
4	0	East Anglia, flat, marginal plains under intensive agriculture.
8	0	England/Scotland coastal / estuarine areas associated with marshes and dunes
12	0	North Midlands Plain, flat very fertile plains.
13	0	Midland Plains, level alluvial plains dominated by intensive agriculture
14	0	North Lowlands of England, level coastal plains.
20	0	South Uplands of Scotland, mid-valley slopes, varied vegetation.
23	0	North-east Highlands of Scotland, high mountain summits, with steep rocky slopes (altitudinally higher than Land Class 24)
31	0	Scotland, far North-west, extremely exposed, peat, coastal.
32	0	Scotland, North-west Isles, extreme, bleak and windswept.

According to the National Vegetation System [170] rowan is a frequent component of ash-rowan (W9) and oak-birch (W17, W16 and W11) woodland communities.

Regeneration

Rowan is a phanerophyte, reproducing entirely by seed which has a requirement for cold, moist stratification to overcome a deep physiological dormancy imposed by both the seed coat and embryo [171]. Some regeneration occurs by epicormic shoots, particularly at high altitude where viable seed is seldom produced, or in response to grazing or coppicing [149, 172]. Lateral clonal spread of up to 5 m from root suckering has occasionally been recorded [172]. Seed dispersal is dependent on the fruit passing through the gut of mammals and birds. Rowan is a pioneer species, able to take advantage of forest openings caused by clear-cutting, forest decline as a result of air pollution and ground disturbance [173, 174, 175]. Despite abundant seedling production, regeneration tends to be limited by grazing owing to its palatability to many mammals including deer, cattle, hares, rabbits and voles who will strip the bark in preference to other tree species [169, 172, 176, 142]. This explains why rowans are often found in inaccessible refugia. Grime [39] cites evidence of allelopathic substances in the leaf litter of rowan which inhibit the growth of some other seedlings, and Edwards and Dixon [175] noted that the dense shade of rowan inhibited regeneration of Scots Pine.

Light

Sorbus aucuparia is adapted to completing its growth cycle within the short growing seasons which occur at high altitude and latitude sites. Håbjørg [55] failed to observe any effect of photoperiod on shoot elongation, although this may have been owing to experimental error (equipment failure). In the same experiment, other tree species (e.g. *Betula verrucosa*), growing at similar latitudes to rowan, had higher shoot

elongation under longer daylength - an adaptation to short growing seasons. Heide [177] observed no response to long days in thermal time to bud burst. Barclay & Crawford[148] recorded a higher relative growth rate (0.149 g g^{-1}) in seedlings raised from seed collected at 560 m altitude in Ballachulish, West Scotland, when grown under standard conditions compared to those collected at 10 m (0.129 g g^{-1}). White [178] measured growth at 560 m altitude in the Pennines, when shoot elongation ceased in mid-August, allowing sufficient shoot hardening before winter.

Rowan is relatively shade tolerant, particularly during the seedling stage. Pigott [179] planted seedlings of *S. aucuparia, Betula pendula* and *Quercus petraea* in pots beneath the dense canopy of *Acer pseudoplatanus*. *Betula* failed to survive; only *Sorbus* demonstrated increased dry mass. Lunde-Høie and Anderson [173] observed in Norway that rowan tends to regenerate in established vegetation close to the mother tree. Similarly, Vanha-Majamaa *et al.* [180] compared rowan regeneration in clear-cut and boreal forest areas in southern Finland. Greatest regeneration was recorded in the forest areas, particularly in the shade of dead trees, although it was also suggested that the dead trees may have provided birds with places to sit and defecate the seeds. In the established phases, rowan trees tolerate partial shade, particularly at low altitudes, although flowering tends to be reduced [181].

Water relations

Linnenbrink *et al.* [143] recorded the bulk water relations of a range of hedgerow shrubs in northern Germany, and classified rowan as a euryhydric species. It showed the greatest diurnal amplitude of water potential (1.9 MPa) and the lowest leaf water potential (-3.0 MPa). Leaf water content depended on the position of the leaf within the canopy. It did not fluctuate as widely throughout the growing season in rowan (80-

120% dry weight) as in *Sambucus nigra* (200% dry weight), which they suggested was owing to a greater allocation of solutes to the leaves in *S. aucuparia*. Although both species were able to tolerate leaf water saturation deficits of more than 40%, damage occurred much sooner in *S. aucuparia* (4-5 hour's desiccation in early summer) than in *Sambucus nigra* (14 hours).

The survival of rowan at high altitudes has been in part attributed to the ability of the winter buds to tolerate winter desiccation [182]. At higher altitudes, the bud scale cuticles were thinner (13.0 μm), less mature and had the greatest decrease in relative water content (19%) than buds at lower altitudes (cuticle thickness 19.3 μm; water content 42%). Despite this, vital staining indicated that the buds were still viable. In a further study, buds of *S. aucuparia* remained viable after 20 days' desiccation in comparison with a range of other tree species including *Quercus robur* and *Fagus sylvatica* which were viable for only 5 days under the same desiccating conditions. It was concluded that the continued survival of *S. aucuparia* at high altitudes must be due to cytoplasmic resistance.

Temperature

The effect of temperature on growth has been little studied. In a multi-variate analysis of height increment with meteorological variables, White [178] observed that shoot elongation occurred below 5.6°C, and that rowan was particularly responsive to the component which he labelled as 'energy' (i.e. temperature, daylength, hours sunshine and relative humidity) and soil temperatures early in the season. Rowan seemed to benefit significantly from cold soil early in the growing season. Barclay [183] suggested that *S. aucuparia* could decrease its dark respiration rate in response to increases in temperature, enabling it to conserve carbohydrate in a low energy environment.

Kronenberg [184] modelled the effect of temperature on flowering date and concluded that it requires a cold period of 750 hours below 7 °C, followed by a temperature sum of 160 day-degrees above a mean day temperature of 6°C, with a base temperature above 6°C.

Nutrition

Gillham [140] suggested that rowan will grow on nutrient-poor soil, but on more fertile soils the growth rate is higher. Foliar analysis of seedling leaves indicated no significant differences between acidic (pH 3.2) or calcareous soils (pH 6.6) : N 5.9 mg^{g-1} ; P 2.8 mg^{g-1}; K 1.2%. Leaf calcium concentrations varied depending on the location of the seedlings: those from an acidic site accumulated less calcium in the leaves (0.5%) irrespective of substrate compared to those from more calcareous sites (1.15%).

Sperens [185] applied mineral fertilisers to a sample of experimental trees and recorded an increased number of flowers, fruit and seed per tree up to 5 years after the fertiliser application compared to control trees. However the fruit-flower ratio per tree did not change, and increased fruit production was owing to increased flower production per tree and inflorescence, rather than any increase in fruit set. The addition of fertiliser increased leaf nitrogen content from 2.5 to 4 mg g^{-1}.

Pollution

Horntvedt [186] studied the accumulation of fluoride from aluminium smelter plants in Norway and observed a linear relationship in rowan between fluoride exposure and accumulation. He calculated the accumulation coefficient (K value) of fluoride accumulation in leaves to a given airborne concentration to be 1.7. Vike and

Häbjørg[187] noted that leaf injury symptoms occurred at fluoride concentrations of 170 ppm and suggested that rowan was a good monitor species of fluoride emissions.

4.6. MYCORRHIZA

Sorbus aucuparia has been recorded in association with both arbuscular mycorrhizal (AM), and less commonly, ectomycorrhizal (ECM) fungi [66]. Studies by Dominik [188] in Poland, and Trappe [189] in the Pacific north-west of America, indicated ECM associations with the imperfect soil fungus *Cenococcum geophilum.* Vosatka [115] recorded AM infection levels of 13-40 % in mining spoil in northern Bohemia where spores of *Acaulospora* spp. and *Glomus* spp. were isolated. Colonisation levels tended to be higher in areas with grass underneath (34-40%) compared to areas without grass (13-35%). Otto and Winkler [190] noted infection levels of 30-60% in orchard soils in Germany. In the United States, Morrison *et al.*[80]observed AM infection levels of 10-20 % in nursery plants. In the same study, inoculation with *Glomus intradices* under high fertility nursery conditions had no effect on growth. No studies appear to have been undertaken to ascertain the mycorrhizal status of rowan in Britain.

4.7. EFFECTS OF SEED PROVENANCE

Few studies have specifically addressed the issue of seed provenance and ecotypic variation in rowan, reflecting a lack of commercial interest in the species. The studies cited below suggest that variation occurs in rowan in response to altitude and soil conditions, and that there may be some genetic basis for this variation which could be exploited. Barclay and Crawford [148] studied the ability of rowan to survive under the conditions of environmental stress associated with altitude and exposure, to determine whether this was due to genotypic or phenotypic factors. Seed was collected along an altitudinal gradient ranging from sea-level to 560 m in Ballachulish, West Scotland.

Significant differences between seed collected at high and low altitudes were recorded in seed weight, viability, stratification requirements and relative growth rates. Seed collected at 560 m weighed less (2 mg) than that collected at 102 m (3 mg); had reduced viability (33% compared to 97%); appeared less mature and required a shorter stratification period: 6-12 weeks compared to 18 weeks. The seedlings which originated from 560 m had a higher mean relative growth rate under standard conditions: 0.149 g g^{-1} day^{-1} compared to 0.129 g g^{-1} day^{-1}, suggesting a adaptation for faster growth in the shorter growing seasons which typify high altitude sites.

Gilham [140] examined ecotypes of rowan adapted to limestone (pH 6.7) and acid (pH 3.9) soils from north Wales, as part of a larger study to select genotypes of amenity trees adapted to difficult landscape sites. Seedlings which originated from the limestone site tended to have a greater shoot height, and root and shoot dry weight, than seedlings which originated on the acid soil, particularly when grown in the limestone soil mix. However this substrate also contained higher levels of nutrients than the acid soil, particularly phosphate: 178 $\mu g\ g^{-1}$ compared to 80 $\mu g\ g^{-1}$. It is worth noting that no consideration was given to the possible role of mycorrhizas to explain these edaphic factors, although this was not within the scope of her study.

Habjorg [55] attempted to consider the effects of latitude in rowan, and observed an earlier cessation of shoot growth was observed in rowan seedlings grown from seed collected at 70°N, compared with seed collected at 56 °N. Popov [191] examined the range of morphological and fruit biochemical traits along a latitudinal gradient from Karelia to the Crimea in a study mainly concerned with improving fruit quality. Tree crown density, fruit dry matter and percentage fruits with dark skins increased along a north-south gradient, while vitamin content declined along the same gradient.

Hillebrand and Rosenberg [192] observed differences based on isozyme analysis between three provenances of rowan (two from north-west Germany, one form Lake Garda, Italy). Genetic diversity, based on 10 loci encoding six enzymes, was also studied by Raspe and Jacquemart [193] between 17 European populations from Finland to the Pyranees. These studies, which give the first evidence of a genetic basis for variation between populations of rowan, suggest that greater exploitation of the silvicultural and landscape potential of this species is possible.

4.8. SEED DORMANCY

Rowan seeds require stratification in moist conditions to overcome a deep physiological dormancy imposed by both the embryo, which requires a period of after-ripening, and the seed coat which may constitute a mechanical barrier to germination. This dormancy has implications for both nursery production techniques as well as seedling regeneration in natural ecosystems.

Flemion [145] conducted exhaustive studies on dormancy in rowan, and observed the beneficial effects of a vernalisation period. Subjecting seeds to 2-4 months at 1°C resulted in 93 % germination, whereas only 29 % germinated at 5 °C. Soaking the seeds in water had a slight effect on germination: 20 % more seeds germinated after 75 days when stratified in a moist compared to dry condition. She also observed that high temperatures induced a secondary dormancy, broken only by repeating the cold treatment. Secondary dormancy was also noted by Razumova 194 when stratification temperatures exceeded 10 °C. Treatment with a mixture of GA_3, thiourea and kinetin accelerated germination at 0-3 °C and prevented secondary dormancy at 10°C. Lenartowicz [195] noted the beneficial effect of a warm period prior to stratification, on synchronising the duration of germination.

Oster *et al.* [196] suggested that compounds in the fruit itself might inhibit germination. Analysis of fruit extracts revealed concentrations of parasorbic acid (4-7 mg g^{-1}), abscissic acid (2.5-3 mg g^{-1}) and isopropylmalic acid (1-1.5 mg g^{-1}) which have been shown to inhibit germination.

The Forestry Commission [171] recommend a period of two weeks at warm, ambient temperature followed by 14-16 weeks cold treatment to overcome dormancy. It is advised that the seed is pre-conditioned for germination by mid-April to avoid a secondary dormancy induced by high temperatures.

4.9. SUMMARY.

Rowan is a hardy native tree, which has a place in many landscape schemes. It is able to tolerate edaphic stresses associated with low nutrient levels, low pH and drought, and is physiologically adapted to altitudinal exposure. It is not, however suited to growing in high pH or waterlogged soils. The small size of its seeds and persistence in the soil allow the rowan to take advantage of regeneration gaps, and its relatively fast growth rate when young and the presence of allelopathic substances in both fruit and leaf litter, give the seedlings some competitive advantage. Rowan also forms a dense canopy which prevents other tree species, such as pine regenerating beneath it. Despite abundant seed production, natural regeneration is limited owing to grazing by a range of herbivores.

Rowan has a wide distribution across a range of habitats in Europe and Asia minor. It appears to interbreed with some other species of *Sorbus*, and there is evidence of a genetic basis for variation in natural populations. However, apart from several decorative and fruit-producing forms, the full potential of this species as a landscape

tree has not been exploited to the same extent as in some other native woodland species such as pine, birch or willow, reflecting a lack of commercial interest. There is also evidence of mycorrhizal associations, but little attention has been given to the nature of the symbiosis and whether this contributes to its survival and fitness.

CHAPTER FIVE.
RESEARCH AIMS AND OBJECTIVES.

5.1. INTRODUCTION

In this chapter the literature review is briefly summarised and the issues highlighted which appear to merit further study. The conclusion of this section will be the generation of a series of null hypotheses to be addressed in the experimental section of the thesis.

5.2. TREE SURVIVAL

There is an unacceptably low level of tree survival on urban landscape sites, the causes of which were identified as the poor quality of planting stock arriving on site, the stress factors which prevail on many sites, and inadequate or inappropriate site management.

Planting stock quality and the importance of a viable and well-configured root system capable of rapid regeneration were emphasised. The issues of physical and physiological damage to stock during the lifting and handling process have already been addressed by previous researchers, and the findings well-publicised, even if not implemented. 'Quality' encompasses the morphological, physiological, genetic and biological aspects of planting stock, which contribute to its ability to survive the transplanting process. Quality is therefore the factor that links the demands of both nurserymen and landscape practitioners. The nurseryman aims to grow a value-added, saleable product, and the landscape practitioner requires a reliable product to realise his/her design and planting intentions, at the lowest possible cost.

Within the context of planting stock quality, there is also potential to match plant root systems to site conditions. Various approaches have been suggested, including:

manipulating cultural practices to improve rooting structure, genotypic selection to exploit natural variation in rooting structure, and inoculation with root symbionts.

5.3. ARBUSCULAR MYCORRHIZAL FUNGI

Arbuscular mycorrhizal fungi can potentially improve plant growth through improvements to plant nutrient uptake, rooting structure and changes in host physiology. There is also evidence that AMF may confer additional benefits in terms of enhanced tolerance of environmental stress. AMF occur in most terrestrial ecosystems but may be absent or depauperate on some landscape sites. Certain nursery and landscape management practices such as choice of substrate, fertiliser and irrigation regime, soil cultivations and pest management, may also reduce the functioning and formation of the symbiosis. AMF species and isolates vary in their ecological preferences and ability to associate beneficially with different taxa of host plants.

Despite much scientific evidence in controlled experimental conditions, the use of AMF has yet to be demonstrated unequivocally under realistic nursery and landscape site conditions. Until this is done nurseries and landscape practitioners are unlikely to be convinced of the merits and costs of adopting mycorrhizal technology.

5.4. CHOICE OF ROWAN AS A CASE STUDY

Rowan was chosen as case study for this thesis because it is a native species, used widely on landscape planting schemes. It is able to tolerate some degree of environmental stress in terms of drought, poor nutrient status and environmental exposure. Rowan is also closely related to many other horticulturally important genera, and so to some extent the findings of this thesis it may be applied to a wider

range of landscape plant taxa. There is some evidence of genetic variation in natural populations, however this has not been exploited in terms of its silvicultural or landscape potential. There is also evidence that rowan forms associations with AMF, but this aspect has not been widely studied.

5.5. RESEARCH AIMS AND OBJECTIVES

This study aims to address both fundamental and applied aspects of the use of arbuscular mycorrhizal fungi to improve plant quality and subsequent establishment in the landscape. The key issues and hypotheses to be explored in the experimental section of the study are indicated in Table 5.1. These are presented according to scientific convention in the form of null hypotheses (H_0) which state that there are no differences between the sampled populations.

Table 5.1. Null hypotheses to be explored in experimental chapters.

Null Hypothesis	Experimental Chapter
H_0 1. Rowan does not associate with AMF	Chapter 7
H_0 2. Soil factors have no effect on the early growth and mycorrhizal infection in rowan.	Chapter 8
H_0 3. Inoculation with AMF has no effect on the early growth of rowan	Chapter 9
H_0 4. All AMF species are equally effective endophytes of rowan.	Chapter 9 and 10
H_0 5. There are no interactions between AMF and host genotype (seed provenance, tree species)	Chapter 9 and 10
H_0 6. Environmental conditions and management practices have no effect on the functioning of AMF.	Chapter 10

CHAPTER SIX
RESEARCH METHODOLOGY.

6.1. INTRODUCTION

This chapter contains details of all of the general methods used in the experimental section including: root staining, assessment of mycorrhizal infection, and statistical analysis. Methods specific to any of the experiments are given in that particular experimental chapter.

A review of experimental methods used in the study of mycorrhiza was undertaken to inform the researcher of potential techniques and their limitations, in order that the most appropriate methods would be selected for the experimental section of this study. The review also highlighted some of the practical difficulties in assessing mycorrhizal infection, which might be encountered by nurseries and landscape practitioners wishing to manage AMF. It was anticipated that the following techniques would be of most relevance: spore extraction; assessments of the level of infective propagules to be found in soils; root staining techniques to indicate whether colonisation has been successful, and assessments of the extent of root infection.

6.2. A REVIEW OF MYCORRHIZAL METHODS

6.2.1. Introduction

The review indicated that most of the techniques used to study mycorrhiza require basic laboratory equipment and skills beyond the scope of many nurseries. The techniques also tend to be time-consuming and initial training and staff supervision may be required. For this reason, an increased demand for laboratories offering diagnostic

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consultancy to nurseries, may be anticipated if mycorrhizas are more actively managed on nurseries and landscape sites.

6.2.2. Spore extraction methods.

Spore extraction methods are used to collect spores for inoculation, as well as to identify and assess the level of indigenous fungi in soils. The main methods of spore extraction from soil have been reviewed by Smith and Skipper [197] , and include wet-sieving and decanting [198]; spore floatation on sucrose solutions [199]; differential sedimentation on gelatine columns [200]; floatation-adhesion [201]; bubbling air through a glycerol-water column [203]; a plate method [197] and centrifuging in a sucrose gradient [114].

Wet-sieving and decanting is a laborious, but effective method for extracting viable spores. It was developed from a technique used to extract nematode cysts and larvae from the soil. Soil samples are suspended in water and sieved to remove large particles of organic matter. The liquid is then decanted, re-suspended in water, sieved using a fine sieve and then transferred to a petri dish for examination. A range of sieve sizes may be used to improve the efficiency of the method and the method may be combined with centrifuging in a sucrose gradient. This method allows a higher number of spores to be recovered and reduces the laborious nature of the task. However the spores must be quickly washed free of the sucrose solution if they are to remain viable. Soil samples are centrifuged in layers of sucrose and water and spores settle into the middle layer where they are removed by pipette.

6.2.3. *Spore identification*

AMF species are identified by their sporocarp, the soil-borne structure containing groups of spores, as well as spore wall features. These include: sporocarp occurrence, shape, colour and size; peridium occurrence and characteristics; spore colour, size and shape; the number of spore walls colour, thickness and ornamentation; hyphal attachment, shape and type of occlusions [203, 204, 205, 206]. Owing to a reliance on spore wall types to separate species Walker [205, 207] proposed a standardised terminology for use in describing spore wall types. A recent taxonomic guide to the classification of AMF is given in Table 6.1.

More recently, molecular studies such as DNA and RNA analysis, isozyme variation, immunological characteristics and wall ultrastructure and cytochemistry have been useful in identifying species [206]. Although these methods are of increasing importance in AMF research, they are beyond the scope of this study.

Table 6.1. Taxonomy of the *Glomales*.
(After Morton and Benny [208]).

Suborder : Glomineae Morton & Benny. Arbuscules and vesicles formed in mycorrhizal roots. Chlamydospores produced terminally or laterally on or within fertile hyphae; auxiliary cells not produced.		
Family	**Genus**	**Definition**
Glomaceae Pirozynski and Dalpé. "Chlamydospores" formed apically from fertile hyphae	*Glomus* Tulasne and Tulasne	Fruiting body a sporocarp; spores also produced singly or in loose to tight aggregates in soil, less commonly in roots.
	*Sclerocystis (*Berkeley and Broome) Almeida and Schenck	Sporocarp composed of spores with lateral walls adherent to one another; connecting hyphae embedded in a central hyphal plexus; chlamydospores in a single layer except at the base; base composed of sterile hyphae
Acaulosporaceae Morton and Benny. Chlamydospores formed from or within the neck of a sporiferous saccule	*Acaulospora* (Gerdemann and Trappe) Berch	Spores arise laterally from the neck of a sporiferous saccule.
	Entrophospora Ames and Schneider	Spores formed in the neck of the sporiferous saccule.
Suborder : Gigasporineae Morton & Benny Arbuscules only formed; azygospoes produced on the apex of a sporiferous cell of a fertile hyphae, auxiliary cell produced		
Family	**Genus**	**Definition**
Gigasporaceae Morton and Benny	*Gigaspora* (Gerdemann and Trappe) Walker and Saunders	Germ tube produced directly through spore wall; inner flexible wall group absent; auxiliary cells finely papillate or echinate.
	Scutellospora Walker and Saunders	Germ tubes form germination shield; inner flexible wall group always present; auxiliary cells knobbly, broadly papillate, or smooth.

6.2.4. Root staining

As indicated in Section 3.3, AM fungi induce little of no macroscopic change in infected roots. The most usual technique to quantify AMF activity is to use a non-vital stain such as trypan blue, acid fuchsin, analine blue or chlorozol black [209, 210, 211, 212, 213]. Other methods include the chitin assay [214, 215], which is a colorimetric method based on the yellow pigment produced in the mycorrhizal roots of some species, and physiological methods such as the succinate dehydrogenase reaction or alkaline phosphatase [75].

A modification of the method of Phillips and Hayman [209] is most often used to detect AMF infections in plant roots. The roots are cleared using potassium hydroxide to remove host cell contents, acidified and then stained. Because of concern over possible harmful effects of the chemicals used, less toxic and simplified methods have been developed substituting methyl blue [210], acid fuchsin [211], or analine blue in acidified glycerol [212] for the trypan blue in lactophenol. The modification of Kormanik et al. [211] and Grace and Stribley [216] are suitable for bulk-processing larger numbers of plant root samples.

6.2.5. Quantifying root infection

The various methods of quantifying infection in stained roots have been reviewed by Becker and Gerdemann [215], Giovannetti and Mosse [217], Biermann and Linderman [218] and Kormanik and McGraw [219]. Assessment is usually based on a presence or absence of infection at root / gridline intersect points, visual estimates of percentage of root cortex infected by fungus, or estimates of length and presence or absence of infection in

root segments mounted on slides. Only Strzemska [220] has made assessments based on transverse sections of roots.

The Gridline-Intersect technique adapted by Ambler and Young [221] expresses infection on a root-length basis. The is based on the calculation of Newman [222] which relates the total length of roots spread out in a given area to the number of times they intersect a series of lines randomly arranged in a given area. A sample of root segments is arranged in a petri dish and the presence or absence of infection assessed at the grid intersection points. Providing that sufficient intersection points are considered, this method gives good results and allows a quantifiable assessment of infection. [217] . Hooker (pers. comm.) recommends a modification of this method in which intersections between the microscope eyepiece graticule and root samples are examined at high magnification to determine percentage infection.

There have been few studies which have considered the minimum level of percentage mycorrhizal infection necessary for beneficial effects. Gemma and Koske [223] demonstrated early growth benefits in American Beachgrass (*Ammophila breviligula*) despite infection levels of only 2-4 per cent. Other researchers have tended to treat percentage infection data with caution, for example St John and Hunt [224] noted variation coefficients of up to 200 per cent between root samples. Reich and Barnard [225] found differences in the proportions of as arbuscules, vesicles and hyphae within the root and also in the amount of external mycelium. This was in agreement with Klironomos [95] who noted differences in the abundance of mycorrhizal structures in different soils and

suggested that fewer arbuscules might represent a reduction in the mycorrhizal functioning.

Several workers have attempted to establish a relationship between the extent of mycorrhizal infection and growth of the host. A direct relationship has been established for ectomycorrhizal species: Last *et al.* [226] observed that seedling height in Sitka Spruce (*Picea sitchensis*) was exponentially related to the number of mycorrhizas. Oliveira *et al* [68] also noted a linear relationship between seedling height in *Pinus patula* and the ectomycorrhizal fungi *Descolea maculata* when percentage mycorrhizal root tips was multiplied by root dry weight. Daft and Nicolson [227] attempted to establish a relationship between the growth of tomato and maize inoculated with species of *Endogone* and concluded that percentage mycorrhizal infection was extremely variable within root systems, although the relationship could be improved by correcting for the size of root system. This is in accord with Gerdemann [228] who observed that plants in fertile soils tended to have more roots than comparable plants in poor soil. It was therefore possible that plants in the fertile soil would have a lower percentage of mycorrhizal roots but a greater total length of mycorrhiza.

6.2.6. Assessing propagule infectivity

Bio-assays are sometimes used as an alternative to spore counts to assess the infectivity of AMF in soils. Wet-sieving and decanting can grossly underestimate the number of propagules by excluding fine endophyte spores and those species which do not produce spores, and does not differentiate between viable and non-viable propagules. Direct spore counts also ignore the contribution of roots, hyphae and vesicles to mycorrhizal

infectivity [229]. The 'most probable number' (MPN) method is a baiting technique [230, 231] in which a series of soil dilutions are mixed with various proportions of autoclaved sand. The soil mixes are then sown with known mycotrophic plants that are grown under controlled conditions and then stained and assessed for infection. One major criticism of the method is that the soil is greatly disturbed, which breaks the hyphae and greatly reduced their infectivity. Another disadvantage is the amount of time required to obtain results of low viability. The method assumes a random distribution of infective propagules within the soil, that every infective propagule is capable of inducing typical mycorrhizas, and that there is no contamination [231]. Estimates are also dependant on experimental conditions, particularly temperature and time of year, which can effect the growth of both host and endophyte [232]. Morton [229] noted some species, notably *Glomus occultum, G. leptochichum, G. fecundisporum, G. tortuosum and G. epigaeum*, which are non-staining and therefore not detected by this assay.

An alternative is the mycorrhizal inoculum potential (MIP) method proposed by Lui and Luo [233] which uses a calculation derived from estimates of the number of spores, vesicles and hyphal connection points per unit length of root.

6.3. MYCORRHIZAL STAINING PROCEDURES USED IN THE STUDY

Roots were stained using a modification of the method of Phillips & Hayman [208] suggested by Koske & Gemma [212] which reduces the use of potentially toxic chemicals without reducing the resolution of the stain. Prior to staining, roots were thoroughly washed to remove any adhering soil or growth substrate, and fixed in a solution of 50%

ethanol to preserve roots and standardise the water content. The chemicals used in the staining processes are given in Table 6.2.

Three versions of the staining process were used in the experimental work:

1. A 'cold' method suggested by Chris Walker (unpublished) which did not prove to be suitable for the plant material, owing to its woody nature and the presence of dense chromatic compounds.
2. A 'hot' method where roots were autoclaved during the clearing and staining process.
3. A bulk processing method to speed up the staining procedure and allow larger numbers of root samples to be handled.

Cold staining method

The root samples were placed in a 2.5% w/v solution of Potassium Hydroxide in a glass universal bottle, at just below boiling point in a fume cupboard for 25 minutes. After allowing to cool, the roots were rinsed once in 2.5% KOH and twice in distilled water. A 3% solution of Hydrogen Peroxide was added, and the roots left for the minimum time necessary for the stele to become clearly visible, before being rinsed in water. A 2% solution of Hydrochloric acid was added and the roots left for at least 1 hour. After rinsing, the roots were stained using acidified Glycerol/Analine Blue (5 ml of 1% Analine Blue in 495 ml 70% acidified glycerol) and left overnight. When required for examination, the roots were de-stained using lactic acid as a slide mount.

Hot staining method.

The roots samples were placed in a glass universal bottle and cleared by autoclaving in a 2.5% aqueous solution of KOH for 3 minutes at 121° C. The roots were then rinsed twice in distilled water and bleached in a freshly prepared solution of alkaline H_2O_2 for approximately 10 minutes or until the roots were paler in colour. It was necessary to bleach the rowan roots as they were still very dark after the KOH treatment. After rinsing, the roots were then acidified in 1% HCL for at least 1 hour, to facilitate stain binding to the fungal structures. The acidified roots were then stained in an acidic glycerol solution, containing 0.05% trypan blue, and autoclaved for 3 minutes at 121° C. The trypan blue solution was then poured off, and the roots de-stained at room temperature in acidic glycerol, in preparation for examination and assessment of mycorrhizal infection. The staining solution, after filtration could be re-used several times.

Table 6.2. Chemical used in root staining.

Stage	**Chemicals required**
1. Root clearing	2.5% aqueous KOH: 25 g KOH pellets in 1000 ml of distilled water.
2. Bleaching	Alkaline H_2O_2: 20 ml NH_4OH, in 80 ml distilled water + 90 ml H_2O_2, made up to 900 ml with distilled water. Solution to be made up fresh, and not stored for more than a week.
3. Acidification	1 % HCL: 10 ml in 990 ml distilled water
4. Staining solution	Acidified glycerol: 500 ml glycerol, 450 ml distilled water, 50 ml 1% HCL To which 0.5 g trypan blue stain is added
5. De-staining	Acidified glycerol (as above, without trypan blue)

Bulk processing

The previous method, while more effective as a stain for rowan roots, was time-consuming owing to the time required in decanting the universal bottles individually between each of the stages. Discussions with other researchers involved in processing large numbers of samples indicted that batch-processing methods were more suitable and less time-consuming. The method suggested by Grace and Stribley [216] involved processing roots in porous containers within a basket which could then be immersed in a sump of the staining chemicals.

Twenty-five ml polypropylene bottles were pierced in the base using a heated needle and fixed into a lidded basket, adapted from an autoclaving basket, with a tightly-fitting mesh-lined lid. The latter was to hold the bottles in place and also to prevent the root samples from being boiled out of the tubes and becoming mixed. Forty tubes could be accommodated within the basket. This was then placed within a large pyrex casserole dish which acted as a sump for the staining solutions.

The staining solutions (1.5 l) were then poured over the tubes, through the lid so that the roots were in good contact with the solutions. A pyrex lid was fitted for the clearing and staining stages which required an autoclave. For rinsing the roots between the various stages, the basket was immersed in a large bowl of distilled water. In this way, 40 root samples could be handled simultaneously.

6.4. QUANTIFYING ROOT MYCORRHIZAL INFECTION.

Percentage infection was determined using a modification of the grid-line intersect method [221, 217] suggested by John Hooker (pers. comm.). A sample of stained roots were placed in a petri dish containing acidified glycerol (500 ml glycerol, 450 ml H_2O, 50 ml 1% HCL). An acetate disc printed with 10 x 10 grid squares, was placed within the eyepiece of the dissecting microscope. Using X320 magnification, the number of roots present at grid-line intersects, and also the presence of AM F structures (vesicles, arbuscules or hyphae) at these intersects were noted. Where there was doubt over the stained structures, these were examined at higher magnifications under a compound microscope. No attempts were made to identify the species causing infection. This was repeated five times for each sample of roots, re-arranging the root segments for each assessment.

Percentage infection per sample was then calculated as:

$$\frac{\text{number of root intersects}}{\text{number of intersects with AM structures.}} \times 100$$

6.5. DETERMINATION OF FRESH AND DRY WEIGHT.

After harvest, the plants were washed and excess moisture removed using paper towels. The shoots and root system were separated at the root collar, and fresh weight

determined. Shoot dry weight was determined by drying the shoots in an oven until no further change in weight was detected.

6.6. PHOTOGRAPHS OF AMF STRUCTURES

All photographs of stained mycorrhizal roots were taken using a Zeiss Axioskop MC80, with Kodak Ektachrome Film.

6.7. STATISTICAL ANALYSIS

All statistical analysis was carried out using SPSS 6.1 for Windows (1994, SPSS Inc.) and Genstat 5, Release 3.1 (1994, Lawes Agricultural Trust, Rothamsted Experimental Station).

The main analyses in the experimental sections used the techniques of Multiple Linear Regression Analysis (MLRA); Analysis of Variance for Split Plot Designs; Least Significant Differences for pair-wise comparisons; Chi-Square, and Logistic Regression.

Multiple Linear Regression Analysis

The data was fitted to a general linear model which comprised the treatment variables for each experiment. This was recommended in preference to conventional analysis of variance techniques due to problems of missing and skewed data distributions that were encountered in the experiments. MLRA assumes that the data has a normal distribution, that the data values are independent of each other, and that there is linearity, in other words that the residuals about a line are normally distributed [234]. However in practice, MLRA is considered to be a more robust statistic for dealing with

deviations from normality and missing values (Bill Adams, pers. comm.). The resulting accumulated analysis of variance assessed the overall influence of the treatment variables used in the experiment to the measures of seedling growth and mycorrhizal colonisation.

Analysis of Variance for Split Plot Designs.

The field experiment in Chapter 10 was arranged in a split-plot design to take account of possible variation in soils, aspect or topography, and also to allow the superimposing of an additional treatment variable (the fertiliser treatment). It was therefore important that the appropriate form of ANOVA was used to analyse the data. While the sum of squares is calculated in the same manner as the standard ANOVA, there is a difference in the manner in which the error terms are calculated. Main plot residuals (in this case the fertiliser treatment) are calculated from plot totals; sub-plot errors from differences between sub-plots in the same plot [235]. For the field experiment (Chapter 10) the complete separation of degrees of freedom is given below in Table 5.3. In the main plots, the mean square is obtained by dividing the sum of squares by the main plot residual; in the sub-plots it is obtained by dividing by the sub-plot residual, which is itself obtained by subtraction. By using this method to calculate the ANOVA, most of the variation due to plots can be removed from the treatment effects of interest. Thus, the treatment effects of tree species and mycorrhiza will be more precisely estimated than that of fertiliser.

Table 6.3. Split-plot analysis of variance for the field experiment

Main plots	**Degrees of freedom**
Fertiliser	2
Residual	6
Sub-plots	
Tree species	2
Mycorrhiza	2
Tree species * mycorrhiza	4
Fertiliser * tree species	4
Fertiliser * mycorrhiza	4
Fertiliser * tree species * mycorrhiza	8
Sub-plot residual	48
Residual	615
Grand Total	647

Logistic Regression

The logistic regression model is a non-parametric test to predict a binary dependent variable (in this case survive/not survive) from a set of independent variables. Unlike ANOVA, logistic regression does not assume that data is normally distributed, and is therefore more appropriate where data has a binomial distribution [234]. The test uses the Wald statistic to test the hypothesis that a coefficient equals zero. The Wald statistic has a Chi-squared distribution, and is the ratio of the coefficient (B) to its standard error. If the significance for a certain variable is less than 0.5, it is concluded that the variable contributes to the equation which classifies cases to either category of the binary dependent variable. However if the coefficient is large, the Wald statistic may be unreliable and it is suggested that a series of models are estimated, with and without that variable, and a X^2 used to determine whether the models are significantly different.

There are several methods for assessing how well the data fits the logistic model:

1. The Classification Table compares the observed outcomes with predictions based on the model and gives an overall percentage of cases correctly classified using the model.
2. Another way of assessing the model is to examine the likelihood of the sample results, given the parameter estimates. It is expressed as two times the log of likelihood (-2LL). If the X^2 is not significant, it can be concluded that the predicted model does not significantly differ from the 'perfect' model.
3. The Goodness of Fit statistic compares the observed probabilities to those predicted by the model. A non-significant X^2 leads to the conclusion that observed and predicted probabilities are not significantly different.
4. The model X^2 refers to differences between the 2LL for the model containing only the constant and the 2LL for the current model with the predicted variables.

The cohort life table.

Gilbertson *et al* [10] used the cohort life table to present data on tree survival on a sample of urban sites in Liverpool. The method, which uses standardised numbers of surviving trees (lx) for each age (x) of the planting cohort, is useful for presenting survivorship ($\log_{10}$ lx), age-specific mortality (qx) and killing power (kx). The latter can be summed meaningfully to give an indication of the total killing power of successive periods. The method is adapted from Law [236] who used it to follow the fate of a cohort of *Poa annua* from initial establishment to death of the last individual, and is described by Begon *et al.* [237].

Life tables enable the pattern of death (and birth) in a population to be studied and compared to other populations. The use of logarithms and standardised population sizes facilitate comparisons between populations of differing sizes. A cohort refers to a group of individuals born, or in this case planted, during the same time interval. Gilbertson *et al* [10] considered that the use of cohort life tables enabled the true death rate of trees to be assessed, as it avoids errors associated with dead trees which are removed from the site or replanted.

All surviving trees are counted at successive monitoring periods; all the column variables are then derived from these values (Table 6.4).

Table 6.4. Derivation of life cohort table.

X	Age (in months)
Ax	Number of trees surviving at each period
Lx	Standardised number surviving (out of 1000)
Dx	Standardised number surviving between x and x+1
Qx	Age-specific mortality rate (dx/lx)
log ax	
log lx	Age specific survivorship
Kx	Killing power (k value) (log ax – log ax+1).

6.8. LOCATION OF FIELD SITES AND SEED COLLECTION

The provenance i.e. geographic areas from which seed used in Experiments One and Two was collected are indicated in Figure 6.1. The sites used to collect root samples (Chapter 7), soils (Chapter 8), and the field planting site (Chapter 10) are indicated on Figure 6.2.

Figure 6.1: Seed Provenances used in Experiments

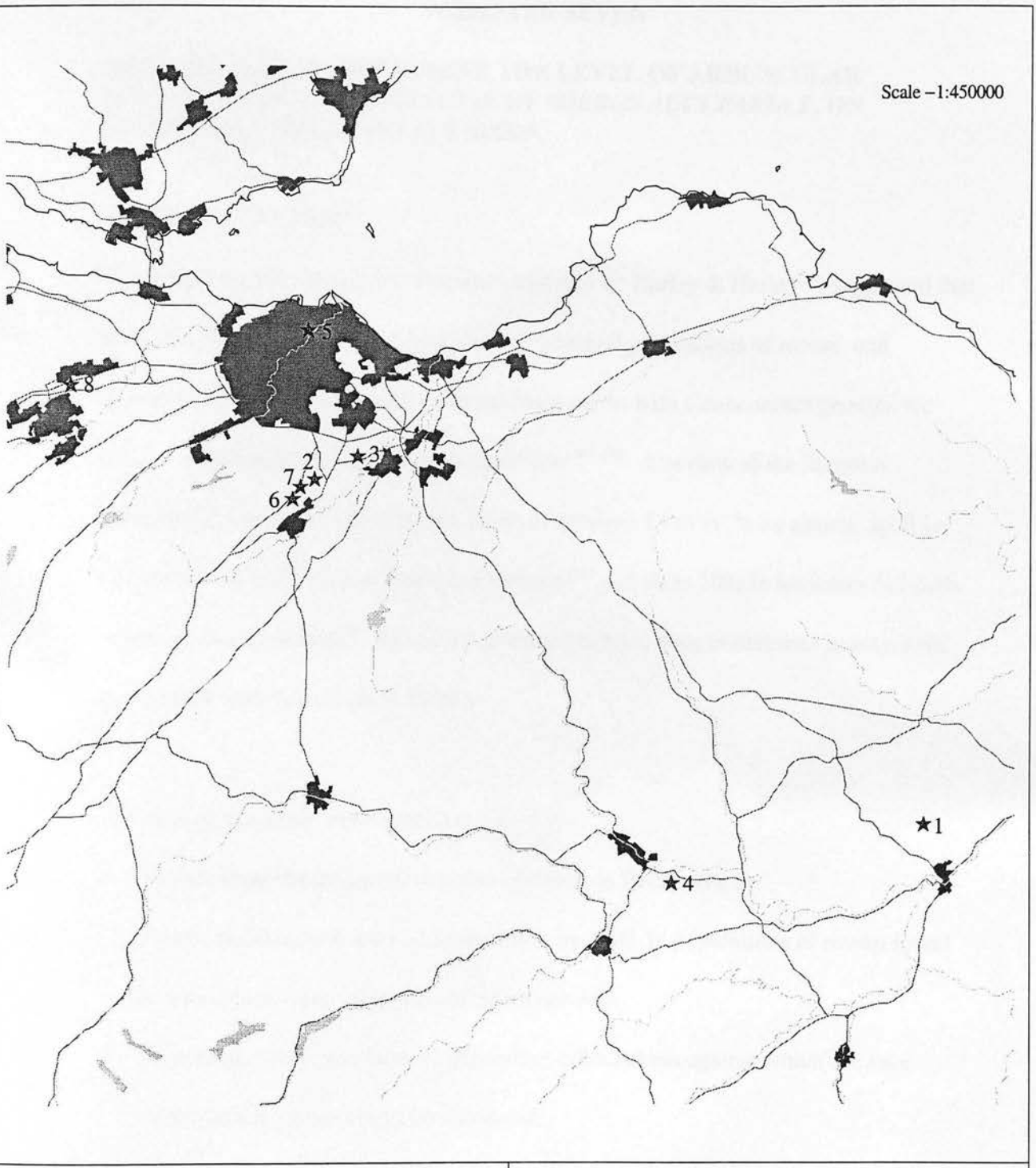
Experiment One (Chapter 8)

1. Bahill
2. Califer Hill
3. Davock
4. Kincardine

Experiment Two (Chapter 9)

5. Pluscarden
6. Loch Naver

Figure 6.2: Sites used in Experimental Studies



Root Samples (Chapter 7)

1. Stitchill Nursery
2. Forestry Commission Nursery
3. Ramsay Bing
4. Sunnyside Farm
5. Royal Botanic Garden Edinburgh

Soil Samples (Chapter 8)

6. Flotterstone
7. Easter Howgate

Planting Site (Chapter 10)

8. Stankards Bing

CHAPTER SEVEN

FIELD STUDIES TO DETERMINE THE LEVEL OF ARBUSCULAR MYCORRHIZAL COLONISATION OF *SORBUS AUCUPARIA L.* ON NURSERIES AND LANDSCAPE SITES.

7.1. INTRODUCTION

The references provided in the checklist complied by Harley & Harley [66], suggested that there was strong evidence of AM infection in natural populations of rowan, and questionable evidence of ectomycorrhizal association with *Cenococcum graniforme* (=*C. geophyllum*) a species of *Fungi imperfecti* [187, 198]. A review of the literature (Section 4.6.) indicated the infection levels of between 13 to 40 % on mining spoil in Slovenia [115] 30 to 60 % in orchards in Germany [190] and 10 to 20% in nurseries in North America (Morrison *et al.*[80]. No studies appeared to have been undertaken to assess the mycorrhizal status of rowan in Britain.

The aims of this study were therefore:

1. To determine the mycorrhizal status of rowan in Britain (H_0 1).
2. To assess the natural level of colonisation by AMF in populations of rowan found in a sample of local nurseries and landscape sites.
3. To provide a reference level of percentage colonisation against which the later experimental studies might be compared.

7.2. MATERIALS AND METHOD

Selection of landscape sites and nurseries

In September/October 1995 a sample of nurseries and landscape architects were contacted for details of landscape sites and nurseries that might be suitable for the collection of root samples. The criteria for the selection of sites were that:

a) they contained a significant planting of native rowan

b) had been planted within the last five years

c) were located within a 50 miles radius of Edinburgh.

Nurseries were selected from a list of those which:

a) advertised in 'Horticulture Week'

b) mostly produced native trees

c) were located within a 50 miles radius of Edinburgh.

Site and nursery details are given below in Table 7.1, and also in Figures 6.1 and 6.2.

Ten plants were randomly sampled from each nursery/site. Roots were excavated using a hand trowel and traced back to the parent tree, in order to verify that the roots were from the intended species. Approximately 5 g of fine roots (less than 2 mm diameter) were removed from each tree. The roots were then washed free of soil or other substrate, and fixed in a solution of 50% ethanol prior to staining for mycorrhizal structures using the 'hot' method (Section 6.3). Visual estimates of percentage mycorrhizal infection were made as described in Section 6.4.

Table 7.1. Location and characteristics of landscape plantings and nurseries sampled for field studies in 1995.

Location	Age of Material (years)	Source of material	Site/nursery characteristics
1. Sites			
Ramsay Bing, Loanhead NT 284 660	5-7	Not known	A rehabilitated coal bing planted with range of native tree species, planted 1991.
Sunnyside Farm,Melrose NT 524 339	2-5	Stichill Forest Nursery	A farm woodlands scheme, planted 1987-1993
Royal Botanic Garden Edinburgh NT 234 628	2	Not known	An 'ecological' area, planted 1994
2. Nurseries			
Stichill Forest Nursery, Kelso NT 714 383	1	Seed collected from local, registered stand	Propagated in mineral seed beds. Source of material for Sunnyside Farm
Forestry Commission, Roslin NT 251 643	1	Not known	'Rootrainer'- grown material, in peat-based substrate.

7.3. RESULTS

Percentage mycorrhizal colonisation for the nurseries and sites sampled are given below in Table 7.2 and Figure 7.1. Figures ranged from 28% (Forestry Commission) to 48% (Sunnyside Farm). One-way analysis of variance indicated statistically significant differences between the groups ($P<0.001$). The means were then compared using Least Significant Differences, which suggested that mean percentage root colonisation in the

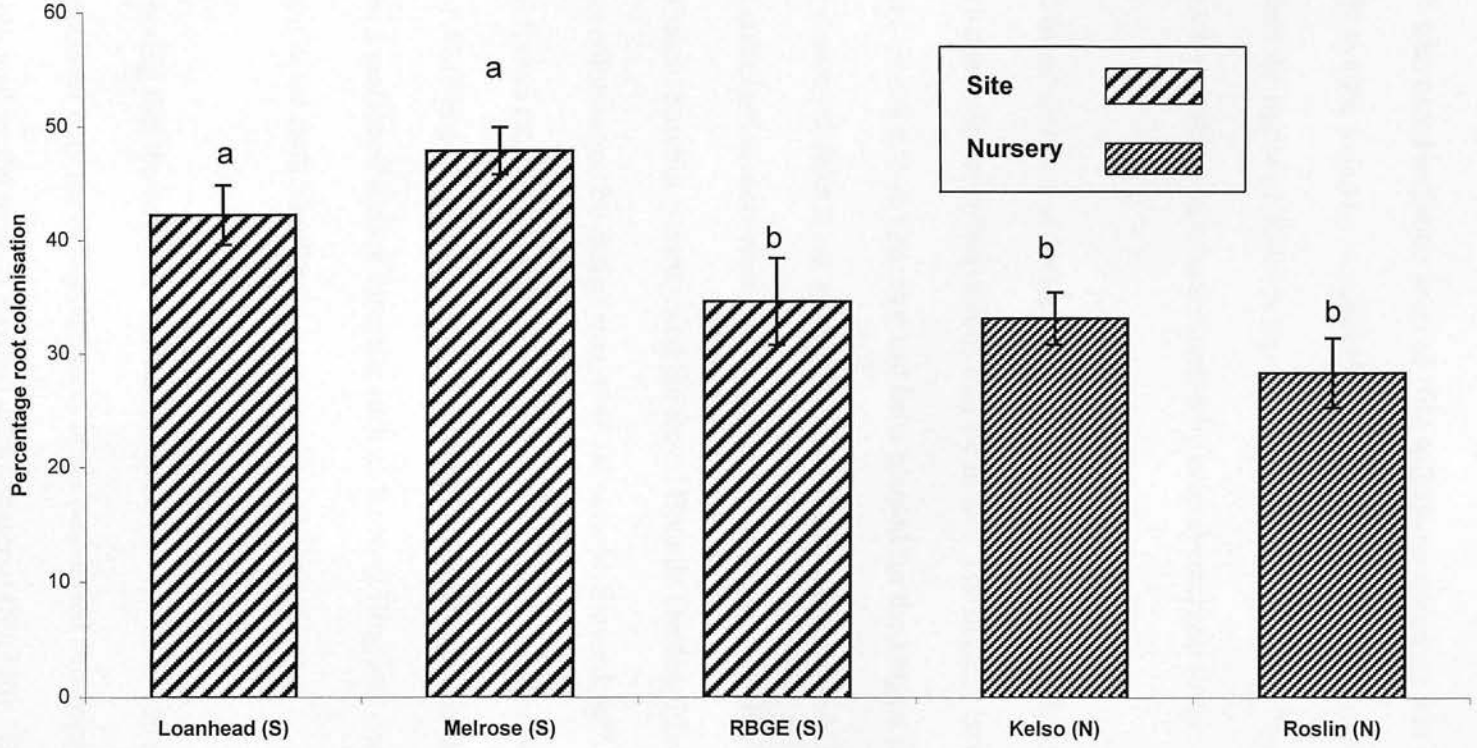
samples from Ramsay Bing (42%) and Sunnyside Farm (48%), were statistically different (at $P<0.05$) than the means of samples from the Forestry Commission (28%), Stitchill Nursery (33%) and the Royal Botanic Garden (35%).

Table 7.2. Percentage mean mycorrhizal colonisation of rowan roots by site.
Figures in brackets indicate standard deviation.

	Site				
	Ramsay Bing	**Sunnyside Farm**	**RBGE**	**Stichill Nursery**	**Forestry Commission Nursery**
	Loanhead	**Melrose**	**Edin-burgh**	**Kelso**	**Roslin**
Sample					
1	35.4	64.5	56.6	39.3	46.0
2	32.5	64.8	38.3	32.6	32.0
3	63.4	27.3	1.7	37.7	31.7
4	41.0	61.0	59.5	31.4	31.0
5	67.5	64.7	72.2	27.5	34.7
6	35.7	35.8	28.7	49.8	5.8
7	29.6	46.0	25.1	23.1	28.6
8	20.7	43.3	34.9	38.9	7.8
9	46.3	12.9	6.0	17.0	27.6
10	52.1	58.0	8.6	32.2	37.3
Mean (SD)	42.2 b[1] (18.5)	47.8 b (21.5)	34.5 a (26.6)	33.0 a (14.2)	28.2 a (16.1)

[1] Means followed by different letters indicate that groups differ significantly $P=0.05$ using least significant differences.

Figure 7.1. Percentage root colonisationby AMF in root samples from landscape sites and nurseries.



7.4. DISCUSSION

This field study has indicated that rowan does form symbiotic associations with AMF on nurseries and sites, and gave an indication of the natural levels of infection. The levels of infection which ranged from 28% up to 48%, compared favourably with Morrison *et al* [80] who noted infection level of 10% to 20% on nurseries and are within the range of 13% to 40% found by Vosatka [115] on sites damaged by acid rain. They are slightly lower than the figure of 30-60 % reported by Otto and Winkler [190]on orchard soils. No evidence was observed of colonisation by ectomycorrhizal fungi.

There were significant differences between the levels of infection from the difference sites, from which a few general observations may be made. The highest levels of colonisation were recorded from sites that had been planted for the longest duration and which therefore contained older trees, namely Ramsay Bing and Sunnyside Farm. The lowest levels of colonisation were recorded on nursery or younger material, namely Forestry Commission, Stitchill Nursery, and the Royal Botanic Garden Edinburgh. This may indicate that colonisation by indigenous AMF takes time: Sieverding[86] suggested that mycorrhizal spread may be less than 1 m per year under field conditions, or the slow recovery of AMF populations following site disturbance. It is interesting to note that after 5 years, a reclaimed coal-mining site such as Ramsay Bing had comparable levels of infection to the farm woodland.

It is also worth noting that the two nurseries sampled had low levels of colonisation; the lowest being associated with the cell-grown trees in the peat-based substrate (Forestry Commission), followed by the mineral seedbed grown material (Stichill). These figures

may reflect the effects of management practices used at the nurseries. Vestburg and Estaun [135] suggested that peat substrates vary in their receptivity to AMF. Lovato *et al.* [88] observed that AMF are very sensitive to soil fumigants, which are known to have been used on the seedbeds. However as no other site parameters were assessed, such as soil pH, nutrient status or moisture, and no quantitative assessments were made of soil infectivity (number of infective AMF propagules) or qualitative assessments of which AMF species were present, it is difficult to speculate further.

Colonisation rates within sites and samples were also extremely variable; the greatest variability being observed in the Botanic Garden, where 3 of the samples had levels of colonisation less than 10%. This variability may in part be attributed to sampling errors within the root system: some of the roots may have been more highly infected, or variation in AMF propagules across sites. Owing to this variability, it is suggested that future experiments should therefore comprise root samples from a large sample of individual trees in order to reduce variation. It is impossible to comment on whether the levels of infection observed in the samples conferred any benefits to the host plant, as no growth comparisons were made between highly infected and poorly infected trees. A higher level of infection does not necessarily infer greater benefits, but may simply indicate an increased carbon drain on the host [137, 72]. As noted by Safir [123] and Sieverding [86] not all AMF species are equally efficient mutualists. Despite these limitations, the results of this study will provide a base level of the level of infection from indigenous soil AMF against which future inoculation experiments may be compared.

CHAPTER EIGHT.
EXPERIMENT ONE: THE EFFECT OF SOIL TYPE AND SEED PROVENANCE ON GROWTH AND MYCORRHIZAL INFECTION OF ROWAN (*SORBUS AUCUPARIA* L.)

8.1. INTRODUCTION

Atkinson and Last [37] suggested that differences in plant growth in different soils might be explained in part by the effect of soil types on the ability of mycorrhizal fungi to establish beneficial associations with host roots. The literature review indicated that there was strong evidence that soil type had a significant effect on AMF colonisation and functioning [97]. Other soil factors which may modify the functioning of the symbiosis include soil pH [86, 106, 107, 108] ; soil moisture and temperature [110, 111] and disturbance [116]. The use of non-sterile soil as a mycorrhizal inoculant has been explored by Sieverding [86]; Wilson *et al.* [120]; Gianinazzi *et al.*[132] and Kemery and Dana [133].

There is also some evidence in the literature of differences in compatibility between host genotype and fungi. Pelham *et al.*[56] reported that seed origin affected the ability of birch to form ectomycorrhizal associations. Similarly, Denny and Wilkins [158] noted that compatibility between fungal strain and host genotype affected the ameliorating influence of the fungi on zinc toxicity. Both these studies were concerned with ectomycorrhizal fungi; few studies appear to have considered the effect of seed provenance and AMF compatibility.

The first experiment was therefore designed to address the following null hypothesis:

1. H_o2. Soil factors have no effect on early growth and mycorrhizal colonisation in rowan.
2. H_o5. There are no interactions between AMF and host genotype.

8.2. MATERIALS AND METHOD

Seed material

In November 1993, the seed of four Scottish provenances of *Sorbus aucuparia* L. were obtained from the Forestry Commission and 'Forestart' (Table 8.1). The location of each seed provenance is also indicated in Figure 6.1. As each seed batch arrived it was exposed to 2 weeks at room temperature before a cold treatment at 1-2°C to overcome any problems with seed dormancy [171]. The seeds were sown in March 1994 in vermiculite, and left to germinate in covered propagation benches in ambient glasshouse conditions. Germination began within one week for most of the seed batches, and no further seedling emergence was recorded after four weeks.

Preparation of soil mixes

Soil samples for the soil inoculation experiment were collected in March 1994 from three sites on the Bush Estate, near Penicuik, where rowan trees were already growing. In this way, it was anticipated that the soils would contain AMF propagules associated with rowan. Details of the sites and their characteristics are given in Table 8.2 and also Figure 6.2.

Table 8.1. Details of seed origin, location, date received and pre-treatment.

Seed Origin	Supplier	Date received	Pre-treatment
Bahill, near Aultmoor NJ 40 53	Forestart	3-12-93	Macerated, extracted and mixed with compost
Califer Hill, near Forres ND 08 57	Forestart	3-12-93	"
Davock NJ 49 51	Forestart	3-12-93	"
Kincardine NO 785 495 / NJ 625 025	Forest Enterprise	24-11-93	Macerated, extracted and mixed with vermiculite

Table 8. 2. Location of soil collection, and site characteristics

Soil	Site and OS Map Reference	Soil Series and Vegetation
1	Flotterston (1) NT 234 628	Winton : imperfectly drained clay loam. Agricultural land sown with Winter Wheat.
2	Flotterston (2) NT 234 628	Winton : imperfectly drained clay loam. Deciduous woodland with deep litter-layer. Rowan, beech, birch and hawthorn.
3	Easter Howgate NT 240 637	Darvil : freely drained clay loam. Stream bank with scattered rowan, ferns and small herbs.

The soils were mixed in equal parts with coarse grit to improve drainage, and half of each sample autoclaved twice at 121^{o}C / 15 lb per square inch for 1 hour, to provide a sterile control treatment (C. Walker, pers. comm.). Sterile and non-sterile samples from each of the soil mixes were sent to SAC, Edinburgh for a basic analysis of pH, P, K and Mg (Table 8.3.). It was considered that these soil parameters would give an indication of soil suitability for tree growth, and mycorrhizal infection. Tree species are known to

vary in the range of pH that they will tolerate; rowan is usually restricted to acidic soils with a pH of less than 5.5 [166]. Phosphorus, potassium and magnesium are all major plant nutrients; phosphorus is associated with early root growth necessary for successful plant establishment; potassium is important for cell division and growth, and magnesium for the formation of chlorophyll [239]. Similarly, it was noted in the literature search, that soil pH and nutrient status have an effect on the abundance of arbuscular mycorrhizal fungi[88].

Table 8.3. Soil origin, soil series and site data.

		Soil Analysis following mixture with coarse grit. Extractable mg/l			
Soil	**Sterile (S) Non- Sterile(NS)**	**PH**	**P**	**K**	**Mg**
1	S NS	5.9 5.6	6.3 (M[1]) 7.2 (M)	145 (M) 112 (M)	175 (M) 171 (M)
2	S NS	4.5 4.5	4.1 (L) 1.5 (VL)	83 (M) 75 (M)	100 (M) 60 (L)
3	S NS	4.3 4.2	4.4 (L) 6.6 (M)	78 (M) 145 (M)	37 (L) 176 (M)

([1] M= moderate level; L = low level; VL = very low status)

Many researchers have noted growth retardation in soils following heat-sterilisation, which they have attributed to the effects of excess ammonia and other soluble salts, or the formation of toxic organic materials [40, 41]. For this reason, the soils were allowed to 'rest' for two weeks following autoclaving to allow dispersal of any of these potential toxins released during autoclaving, before use in the experiment.

The seedlings were potted into 1.1 litre pots containing the soil mixes in May 1994 and arranged in two plots on the same glasshouse bench. There were with 8 plants of each seed provenance randomised within each soil treatment i.e. n = 8 (Figure 8.1). This gave a total of 192 experimental plants.

Figure 8.1. Experimental Design for Experiment One

Glasshouse wall											
Plot A						Plot B					
Soil 1		Soil 2		Soil 3		Soil 1		Soil 2		Soil 3	
S[1]	NS[2]	S	NS	S	NS	S	NS	S	NS	S	NS
Glasshouse interior											

[1] Soils sterilized by autoclaving
[2] Soils not sterilized.

During the first few weeks, many of the seedlings planted into the sterile soils failed to establish and died, perhaps as a result of toxic substances which continued to be released from the autoclaved soils. These soils were then flooded to excess, and replanted. Measurements were made at the end of each growing season of height to apical bud (in mm) and leaf number. The number of surviving seedlings was also noted. When the experiment was terminated in June 1996, a random sample of plants were harvested and stained to detect the presence of mycorrhizal structures in the roots (Section 6.3 and 6.4).

8.3. RESULTS

8.3.1. Statistical analysis.

To overcome the problem of skewed distributions (which were not improved by logarithmic transformations) and missing data resulting in an unbalanced design, Multiple Linear Regression Analysis (MLRA) was used to assess the overall influence of the treatment variables used in the experiment. Differences between treatment means were explored using Least Significant Differences (Section 6.10). An explanation of the treatment factor levels and dependent variables is given in Table 8.4.

Table 8.4. Treatment Factor Levels and Dependent Variables

The treatment factors :

- Soil : 3 levels (Soil 1, Soil 2, Soil 3)
- Sterile : 2 levels (Sterile, Non-sterile)
- Seed : 4 levels (Bahill, Califer Hill, Davock, Kincardine)

The dependent variables :

- Height (in mm) in 1994, 1995, 1996
- Height Change (1994-1995, 1995-1996)
- Leaf number (1994, 1996)
- Change in leaf number (1994-1996)
- Percentage infection (1996)

The resulting accumulated analysis of variance derived from MLRA is shown in Table 8.5. below. As may be inferred from this table, there were no significant 3-way interactions between the factors Soil, Sterile and Seed. The only significant 2-way interaction was between Soil and Sterile, which was significant for height and leaf number (years 1994 to 1996) but not significant for percentage mycorrhizal infection. Mycorrhizal infection in 1996 was affected by the main treatment effect of the soil sterilisation treatment only. Seed provenance was not significant as either an

Table 8.5. Overall accumulated analysis of variance (F-Statisitic; degrees of freedom, significance).

	Height 1994	Height 1995	Height 1996	Height change 1994-1995	Height change 1995-1996	Leaf number 1994	Leaf number 1996	Increases in leaf number 1994-6	Percentage Infection 1996
Main Effects									
Soil	**28.27, 2 ***[1]**	**25.58, 2 *****	**50.30, 2 *****	**20.83, 2 *****	**24.07, 2 *****	**41.65, 2 *****	**25.23, 2 *****	2.60, 2 ns	2.95, 2 ns
Sterile	1.44, 1 ns	**5.86, 1 ***	**8.82, 1 ****	**5.70, 1 ***	2.76, 1 ns	2.03, 1 ns	**17.22, 1 *****	**4.55, 1 ***	**17.19, 1 *****
Seed	2.51, 3 ns	0.58, 3 ns	1.72, 3 ns	0.55, 3 ns	2.48, 3 ns	1.41, 3 ns	0.53, 3 ns	0.53, 3 ns	0.40, 3 ns
2-way interactions									
Soil* Sterile	**3.33, 2 ***	**6.20, 2 ****	**14.17, 2 *****	**5.89, 2 ****	**8.38, 2 *****	**5.33, 2 ****	**8.04, 2 *****	0.34, 2 ns	2.62, 1 ns
Soil* Seed	1.30, 6 ns	0.85, 6 ns	0.83, 6 ns	0.79, 6 ns	1.31, 6 ns	0.91, 6 ns	0.62, 6 ns	0.36, 6 ns	1.33, 2 ns
Sterile*Seed	1.38, 3 ns	0.58, 3 ns	0.44, 3 ns	0.36, 3 ns	0.71, 3 ns	0.79, 3 ns	1.24, 3 ns	1.70, 3 ns	0.39, 2 ns
3-way interaction									
Soil*Seed *Sterile	0.67, 6 ns	0.15, 5 ns	0.41, 4 ns	0.16, 4 ns	0.24, 4 ns	1.11, 6 ns	0.19, 4 ns	0.09, 4 ns	-[2]

[1] ***Very highly significant at P<0.001; ** highly significant at P<0.01, * significant at P<0.05, ns = not significant.

[2] Impossible to calculate due to missing values.

interaction or main treatment effect, and so all the provenances will be combined in the presentation of results.

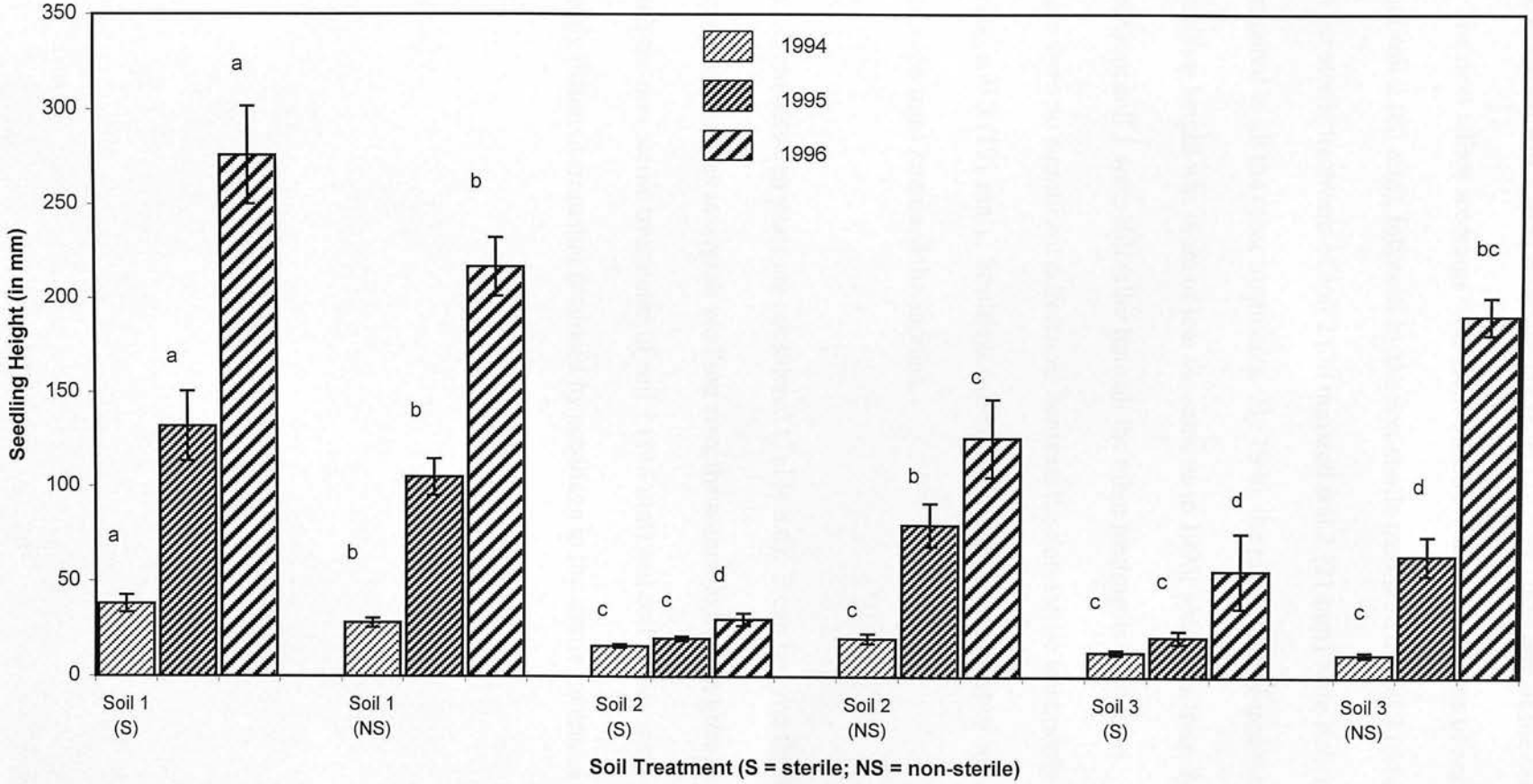
8.3.2. Seedling height.

There was a significant interactive effect between the soil and sterile treatments in all years: with the notable exception of soil 1, seedlings were consistently taller on the non-sterile treatments than the sterile treatments. This is indicated in Table 8.6 and also Figure 8.2.

Table 8.6. The effect of soil type and soil sterilization on seedling height (in mm).
Figures in parenthesis indicate standard error and sample size (n).
Within year treatments followed by different lower case letters differ significantly using LSD at P>0.05.

	Height by year			Height change	
Treatment	**1994**	**1995**	**1996**	**1994-5**	**1995-6**
Soil 1					
1.Sterile	38 a (4.6, 30)	132 a (18.5, 21)	276 a (25.8,20)	92 a (13.8,21)	143 a (20.3, 20)
2. Non-sterile	28 b (2.5, 31)	105 b (9.7, 22)	217 b (15.6,22)	77 a b (9.1, 22)	114 a (13.1,21)
Soil 2					
3. Sterile	16 c (1.0, 27)	20 c 1.2, 17	30 d 3.2, 17)	4 d (1.0, 17)	10 c (2.6, 17)
4. Non-sterile	20 c (2.6, 30)	80 b (11.5,13)	126 c (20.7, 12)	57 b (8.2, 13)	57 b (8.1, 13)
Soil 3					
5. Sterile	13 c (1.4, 23)	21 c (3.4, 15)	56 d (19.8, 13)	7 d (3.0, 11)	34 b (19.4, 13)
6. Non-sterile	12 c (1.4, 21)	64 d (10.2, 6)	191 b c (9.9, 6)	49 b (8.8, 6)	126 a (5.6, 6)

Figure 8.2. The effect of soil treatment on seedling height (in mm) 1994-96.
(Error bars apply within years only)



In 1994 the tallest seedlings were those grown in the sterile (38 mm) and non-sterile (28 mm) treatment of soil 1, followed by all the other soil treatments. In 1995 seedlings grown in the sterilised soil 1 were considerably taller (132mm) than any of the other soil treatments. The next tallest seedlings were from the non- sterile treatments of soil 1 (105 mm) and soil 2 (80 mm), followed by the non-sterile treatment of soil 3 (64 mm). Seedlings in the sterile treatment of soil 2 (20 mm)and soil 3 (21 mm) were noticeably dwarfed compared to all the other treatments. By 1996, the rank order of treatments effects on seedling height was more or less the same as in 1995; seedlings from the sterile treatment in soil 1 were still taller than all the other treatments (276 mm). However there were no significant differences between the non-sterile treatments in soil 1 (217 mm) and soil 3 (191 mm). Seedlings grown in the sterile treatment of soil 2 (30 mm) and soil 3 (56 mm) remained the shortest.

If changes in height between years are considered (Table 8.6), it can be seen that by 1995-96, despite differences in overall seedling size, the annual extension growth of seedlings from the non-sterile treatments of soil 1 (114 mm) and soil 3 (126 mm) was not significantly different from that produced by seedlings in the sterile treatment of soil 1 (143 mm).

8.3.3. Leaf number.

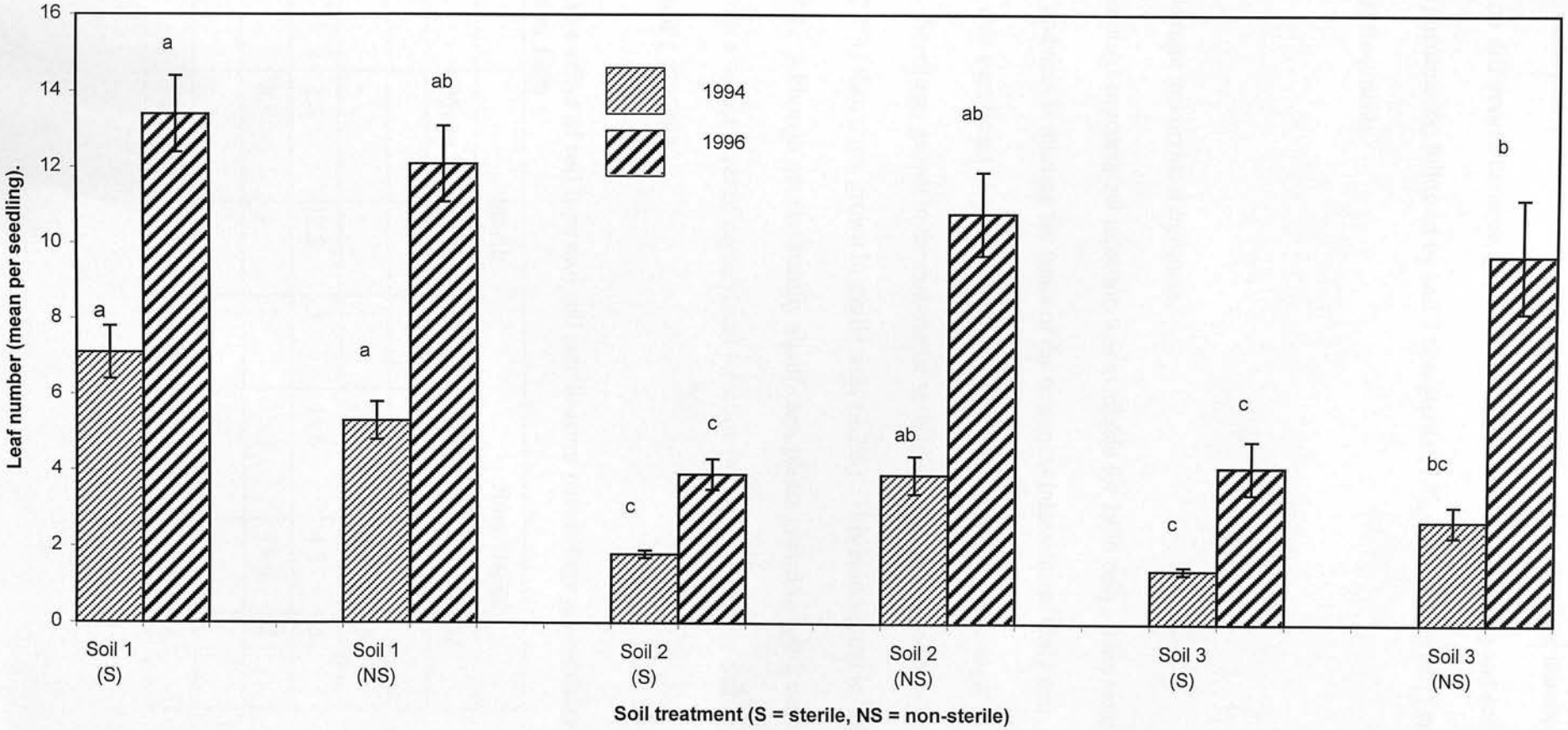
Leaf number was strongly influenced by the 2-way interactive effect of the soil type and soil sterilisation treatments. This is indicated in Table 8.7 and Figure 8.3.

Table 8.7. The effect of soil type and soil sterilisation on leaf number (mean per seedling) in 1994 and 1996.
Figures in parenthesis indicate standard error and sample size (n). Treatments within year followed by different lower case letters differ significantly using LSD at P>0.05.

	Leaf number (1994)	Leaf number (1996)	Increases in leaf number (1994-96)
Treatment			
Soil 1			
1. Sterile	7.1 a (0.7, 30)	13.4 a (1.0, 20)	5.8 a (1.2,20)
2. Non-sterile	5.3 a (0.5, 31)	12.1 ab (1.0, 22)	6.6 a (1.1,22)
Soil 2.			
3. Sterile	1.8 c (0.1, 26)	3.9 c (0.4, 17)	2.3 c (0.4, 17)
4. Non-sterile	3.9 ab (0.5, 30)	10.8 ab (1.1, 12)	5.8 a (1.1, 12)
Soil 3.			
5. Sterile	1.4 c (0.1, 23)	4.1 c (0.7, 13)	1.6 c (0.4, 10)
6. Non-sterile	2.7 bc (0.4, 21)	9.7 b (1.5, 6)	4.8 bc (1.8, 6)

As in the height parameter, with the exception of soil 1, the greatest number of leaves per seedling occurred in the non-sterile soil treatments. In 1994, there were no significant differences in leaf number between seedlings grown in the sterile (7.1) or non-sterile (5.3) treatment of soil 1, and also the non-sterile treatment of soil 2 (3.9). This was followed by the non-sterile treatment of soil 3 (2.7) and the sterile treatments of soil 2 (1.8) and soil 3 (1.4).

Figure 8.3. The effect of soil treatment on leaf number(mean per seedling) in 1994 and 1996.
(Significant differences between treatments are indicated by different lower case letters and apply within year only)



In 1996, the same rank order of treatments was recorded in respect of leaf number. There were no differences between soil 1 sterile (13.4), non-sterile (12.1) and soil 2 non-sterile (10.8) treatments; followed by soil 3 non-sterile (9.7), soil 3 sterile (4.1) and soil 2 sterile (3.9) treatments.

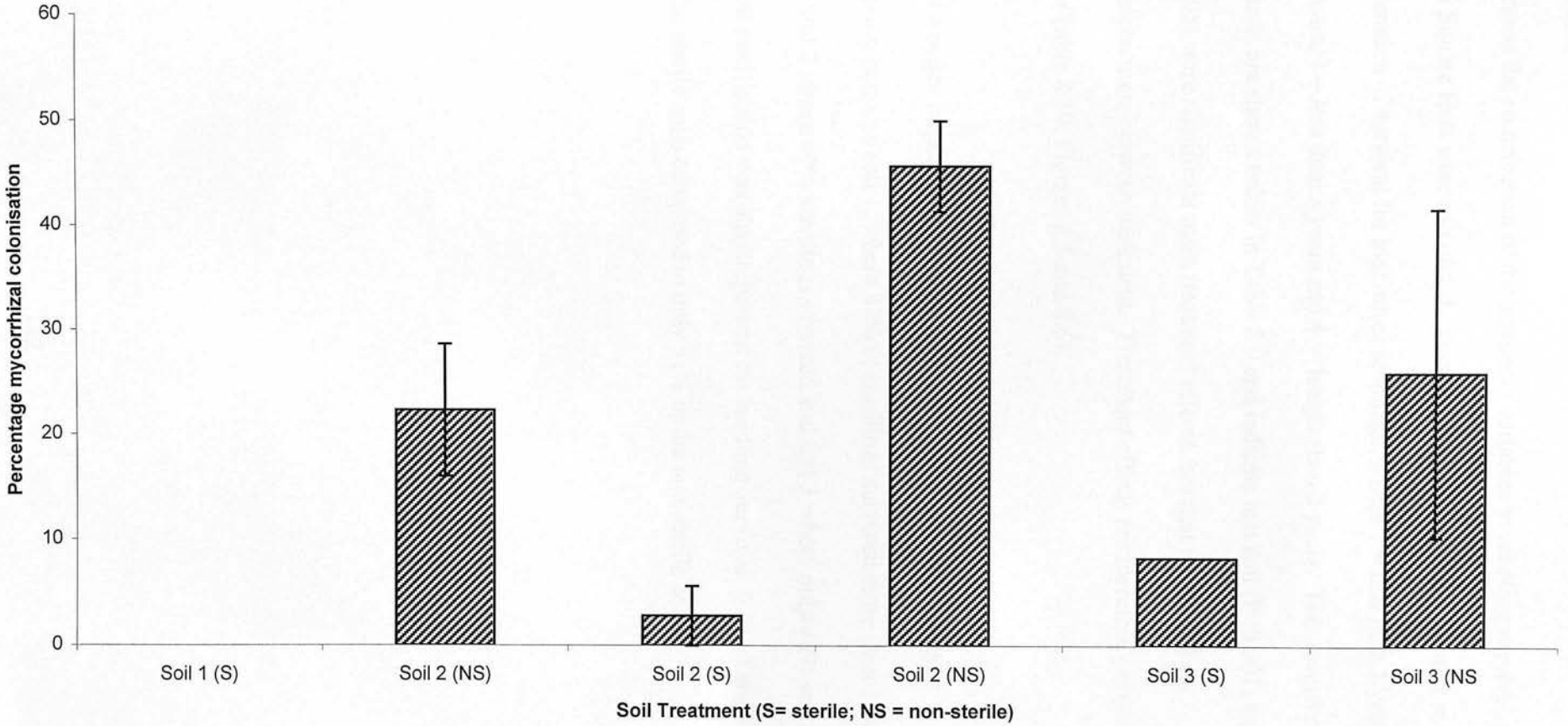
8.3.4. Percentage mycorrhizal infection.

Data on percentage mycorrhizal infection was available for 1996 only. This sample was incomplete, and data is missing for some of the treatment interactions. Only soil sterilisation was significant as a main treatment effect; there were no treatment interactions. Seedlings grown in the non-sterile soils had a higher level of mycorrhizal infection (32.5%) than those grown in sterile soils (4.2%). This is indicated in Table 8.8 and Figure 8.4. Although not statistically significant, plants grown in soil 2 were associated with a higher level of mycorrhizal infection (45.6%) than either soil 3 (25.9%) or soil 1 (22.2%).

Table 8.8. The effect of soil type and soil sterilisation percentage mycorrhizal colonisation in 1996

	Sterile			**Non-Sterile**		
Soil type	**Mean**	**S.E.**	**N**	**Mean**	**S.E.**	**N**
Soil 1	-	-	-	22.3	6.3	5
Soil 2	2.8	2.8	3	45.6	4.3	5
Soil 3	8.3	-	2	25.9	15.6	2
Total	4.2		5	32.5		12

Figure 8.4. The effect of soil treatment on percentage mycorrhizal colonisation in 1996



8.3.5. Seedling survival.

In order to assess the contribution of the treatment variables to seedling survival, a series of Chi Square tests were calculated. Survival was scored on a scale of 1 to 4 to denote the duration of survival for individual seedlings, where 1 = less than 1 year, 2 = less than 2 years, 3 = less than 3 years and 4 = longer than 3 years. The results of the Chi-Square tests are shown below in Table 8.9 and indicate that soil ($P<0.001$) and sterile ($P<0.05$) were significant main treatment effects, but that there were no significant interactions between treatment. Treatment effects are therefore presented individually (Table 8.10; Figures 8.5 and 8.6).

Soil type had a major impact on seedling survival ($P<0.001$), with the greatest differences being between soil 1, where 82% of seedlings survived more than 3 years, compared to soil 2 where 60% seedlings survived and soil 3 where only 40% seedlings survived. Soil sterilisation was also important for seedling survival: 69% of seedlings survived in the sterile soils compared to only 51% in the non-sterile soils.

Table 8.9. Chi-square tests on seedling survival: the contribution of soil type, soil sterilisation and seed provenance.

Source of Variation		Chi Square	DF	Significance
1. Soil	Total soil	17.11	2	***[1]
	Soil 2 v soil 1	0.04	1	ns
	Soil 3 v soil 1	17.06	1	***
	Soil * seed	7.63	6	ns
	Soil * sterile	2.93	2	ns
	Soil * seed * sterile	7.84	6	ns
2. Sterile	Sterile v non sterile	4.06	1	*
	Sterile * soil	3.34	2	ns
	Sterile * seed	6.17	3	ns
	Sterile * soil * seed	7.10	6	ns
3. Seed	Total seed	7.55	3	Ns
	Seed 2 v seed 1	5.32	1	*
	Seed 3 v seed 1	1.24	1	ns
	Seed 4 v seed 1	0.98	1	ns
	Seed * soil	8.57	6	ns
	Seed * sterile	6.08	3	ns
	Seed * soil * sterile	8.52	6	ns

[1] *** = Very highly significant at P<0.001, ** = highly significant at P<0.01, * = significant at P<0.05, ns = not significant

Table 8.10. The effect of soil type, soil sterilization and seed provenance on the duration of seedling survival (in years).

		Number of seedlings which survived				
		< 1 year	1-2 years[1]	2-3 years	> 3 years	Total [1]
Soil	1	4	1	4	39	48
	2	6	12	1	29	48
	3	12	15	2	19	48
Sterile		9	10	3	50	72
Non-Sterile		13	18	4	37	72
Seed	Bahill	3	5	0	28	36
	Califer	10	8	1	17	36
	Davock	6	7	2	21	36
	Kincardine	3	8	4	21	36

[1] Figures adjusted to allow for seedlings (a sample of 2 plants from each treatment combination) destructively harvested at the end of 1994

Seed provenance did not have a significant overall effect on survival, although there were differences between Bahill, where 78% seedlings survived, compared to Califer Hill where only 47% seedlings survived. These figures may have been affected by the high level of seedling mortality in the Califer Hill provenance recorded in the non-sterile treatments of soil 2, where only 1 seedling survived and soil 3 where no seedlings survived.

Figure 8.5. The effect of soil treatment on percentage seedling survival after 3 year.

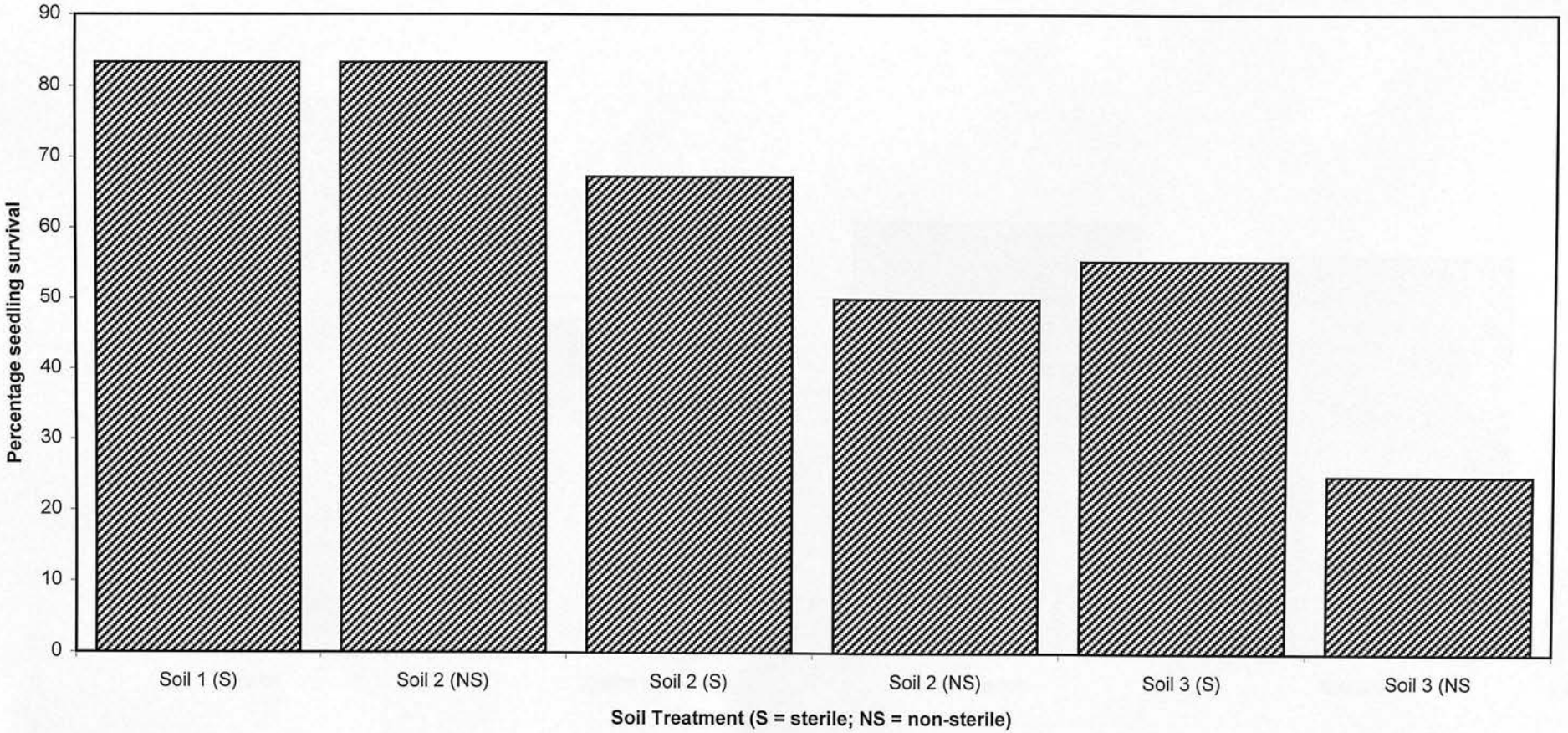
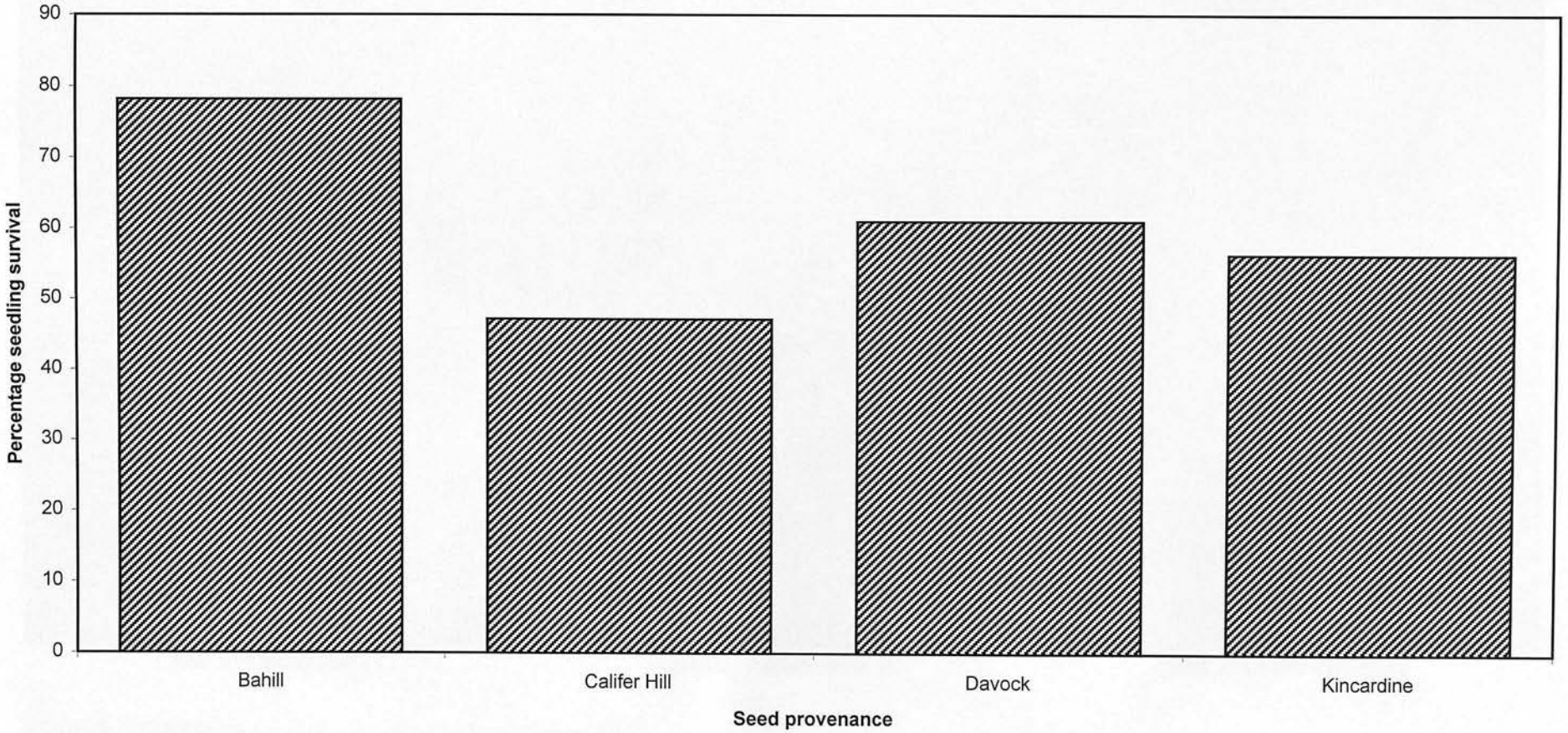


Figure 8.6. The effect of seed provenance on seedling survival



(Soil 1) Flotterstone 1. (Soil 2) Flotterstone 2. (Soil 3) Easter Howgate

Figure 8.7 Sites used for soil collection in Experiment One

Figure 8.8 Experimental plants in September 1994 (Sterile treatments)

Soil 1 Sterile

Soil 2 Sterile

Soil 3 Sterile

Figure 8.9 Experimental plants in September 1994 (Non -Sterile treatments)

Soil 1 Non –Sterile

Soil 2 Non –Sterile

Soil 3 Non -Sterile

8.4. DISCUSSION

8.4.1.Introduction.

The aims of this experiment were to explore the effects of soil type and seed provenance on the growth and survival of rowan seedlings, and to attempt to relate these effects to mycorrhizal colonisation. A factorial pot experiment was therefore set up comprising of soil type (3 levels), soil sterilisation (2 levels) and seed provenance (4 levels). A preliminary literature review suggested that soil type had an effect on mycorrhizal colonisation[80, 95], and that the ability to form mycorrhizal associations was affected by seed origin [56, 58].

8.4.2. *Summary of treatment effects.*

1. There was a significant interaction effect between soil type and soil sterilisation on seedling height and leaf number in each of the years that the experiment was monitored: seedlings grown in soil 1 were consistently taller and had more leaves than those grown in soil 2 or soil 3. In soil 1, seedlings grew better on the sterilised soil; in soil 2 and soil 3, seedlings grew better in the non-sterile soil. However, by the end of the third year, there were no differences in annual shoot extension between seedlings in soil 1 (sterile and non-sterile treatments) and the non-sterilised treatment of soil 3.
2. Soil sterilisation had a significant effect on percentage mycorrhizal colonisation: levels of mycorrhizal colonisation were higher in the non-sterile (32.5%) compared to the sterile soil treatments (4.2%).
3. Seed provenance had no main treatment or interactive effects on height, leaf number or mycorrhizal colonisation, but had a significant effect on the duration of

seedling survival for two of the provenances: 75% of the Bahill seedlings survived for more than 3 years, only 47% of those from Califer Hill

4. Soil type had significant main treatment effects on seedling survival: a greater number of seedlings survived more than 3 years in soil 1 (82%) than in soil 2 (60%) or soil 3 (40%).
5. Soil sterilisation had a significant main treatment effect on seedling survival: a greater number of seedlings survived more than 3 years on the sterile soils (69%) than on the non-sterile soils (52%).

8.4.3. Effect of soil treatment on growth and survival (H_O2)

Mycorrhizas are symbiotic interactions between plants and fungi, mediated by soil factors [137]. These soil factors include nutrient status, pH and soil type, which were assessed at the start of the experiment, as well as AMF propagules and pathogenic organisms present in the soil, which were not assessed. The soils in the experiment were also subjected to disturbance (removal from site, mixing with grit and potting up).

Soils 1 and 2 were both from the Winton soil series, which comprise imperfectly-drained clay loams, and soil 3 was from the Darvil soil series which comprise free-draining clay loams. Soil 1 was on agricultural land sown with winter cereals at the time the soil samples were taken, and soil 2 was under semi-natural woodland containing rowan, beech and birch trees, with a deep organic litter layer. Soil 3 was a stream bank vegetated with rowan, ferns and other small herbs. Soil 1 had the highest pH (5.7) compared to soil 2 (pH 4.5) and soil 3 (4.3). Soil 1 also had higher levels of phosphorus, potassium and magnesium than either soil 2 or soil 3.

Rowan is usually restricted to acidic soils with a pH level of 5.5 or less [166], although ecotypes adapted to limestone soils have been observed [140]. Some of the variation between soil types may be explained by pH: although soil 1 had the highest pH (5.6-5.9) the provenances used in this experiment may have been better adapted to this pH than the more strongly acidic conditions (pH 4.2-4.5) recorded in soils 2 and 3. In addition, nutrient availability to plants tends to decrease in acidic conditions. All the soil nutrient levels for soil 1 were within the moderate range, whereas some of these nutrients in soils 2 and 3 were within the low or very low range. Another suggestion might therefore be that the effects of soil type may be explained in terms of nutrition : the rowan seedlings grew better on more fertile agricultural soils.

There was also an interesting relationship between seedling survival and growth: seedling survival was not always related to the soil treatment that produced the most shoot growth. It is possible that some of the soil treatments exerted a selection pressure on the rowan seedlings, such that those which survived were in some way fitter and more adapted to the treatment soil conditions. By the end of the third year, the six seedlings which persisted in the non-sterile treatment of soil 3, produced a similar amount of extension growth to the best treatments in soil 1. However the 17 seedlings which persisted in the sterile treatment of soil 2 had barely produced any extension growth over the three years. This would suggest that the factors that govern seedling survival in rowan differ from those that determine growth.

The soil sterilisation treatment was undertaken to provide 'control' soils with no mycorrhizal propagules, however the treatment itself also had an effect upon seedling growth and survival, soil nutrient levels and pH. In the initial stages of the experiment, some seedling mortality occurred in the sterile soils which may have been attributable to a release of toxic substances associated with soil heat [240, 241]. However, this problem disappeared when the affected soils were flooded to excess and re-planted. As indicated earlier (Table 8.3) soil sterilisation raised pH slightly in soil 1 and soil 3, but had an inconsistent effect upon soil fertility, for example the level of soil phosphate was increased by heat sterilisation in soil 2 but decreased in soil 3.

The interaction between soil type and soil sterilisation affected seedling growth: although plants in soil 2 and soil 3 grew better in the non-sterile soil, they did not grow as well as the soil 1 plants, which grew better in the sterile soils. There are two possible explanations of these results: either heat sterilisation was more injurious to the soil chemistry of soils 2 and 3 which contained higher amounts of organic matter prior to autoclaving. Alternatively, in the lower nutrient levels of soils 2 and 3, the micro-organisms present in the non-sterile soils had a beneficial effect on rowan, but that in the more higher nutrient levels soil 1, growth was inhibited by the presence of contaminating micro-organisms or by ineffective symbionts. Although not recorded in the experiment, it was observed that many of the roots from the non-sterile soils were contaminated by chytrid-type fungal organisms (Roy Watling, pers. comm.).

8.4.4. Effects of soil treatment on mycorrhizal colonisation (H_o2).

Mycorrhizal colonisation was assessed once at the end of the experiment, in May 1996, and so the results are only representative of the sample taken at that time of year. Not surprisingly, autoclaving the soil had a significant effect on mycorrhizal colonisation: the level in the sterilised soils was less than 5%; the level in non-sterilised soil more than 30%. That any infection occurred in the autoclaved soils may be explained by cross-contamination of mycorrhizal fungi within the nursery or irrigation water.

Levels of colonisation varied between soils, although this was not statistically significant: 22.5% in soil 1, 25.9% in soil 3 and 45.6% in soil 2. These were within the range of 28-48% observed in the field survey (Section7). It is interesting to note that levels of mycorrhizal colonisation associated with the greatest seedling height varied between soils: on soil 1 height was maximised at 32% colonisation; in soil 2 height was maximised at 55%. This suggests either that the AMF populations in soil 1 were more efficient at promoting host growth or, that mycorrhizal colonisation was in some way regulated by the host according to the nutrient status of the soil and host dependency on AMF.

In some ways, soil 2 may be considered as the 'unimproved' version of soil 1. This finding would therefore be in accordance with Johnson and Pfleger [137] who noted that AMF population diversity declined when natural ecosystems were converted to agriculture owing to the increasing intensity of land management, for example chemical inputs and soil cultivations. These practices have been shown to exert selection pressures on AMF populations, as well as reduce soil infectivity by disturbing the

mycelial network and changing the spatial distribution of propagules in the soil. However, bearing in mind the small number of plants sampled for mycorrhizal colonisation in this experiment, and the absence of any assessments of soil AMF populations, it is unwise to speculate further. The effects of land and nursery management practices on AMF populations and functioning would appear to warrant further attention.

It has also been suggested that soil fertility and in particular high soil phosphate levels can inhibit mycorrhizal infection [84] or at least preclude mycorrhizal benefits [105, 237, 72]. The locations from which soils 2 and 3 were collected had rowans already present and so might be expected to contain micro-organisms which associate with rowan. In contrast, the characteristics of soil 1 may have selected for soil micro-organisms adapted to agricultural crops. It is also possible that the AMF propagules in soil 2 were more tolerant of the disturbances caused during soil collection, mixing and potting.

These finding are also in line with those of Morrison *et al.*, [80] who observed that under nursery conditions of high fertility, rowan established commensal or even parasitic relationships with AMF. Hayman [105] proposed that, under optimal phosphate conditions, plants with an extensive and fibrous root system can obtain sufficient soil resources and are less likely to benefit from the association, compared to plants which have a coarser, less fibrous root system. Rowan has an extensive and fibrous root system. The results from this experiment would appear to suggest that under favourable soil conditions, the growth of rowan responds more to increased nutrient levels than

mycorrhizal association, and that under higher nutrient conditions, growth may be inhibited by the presence of soil micro-organisms.

8.4.5. Effects of host genotype (H_O5).

Seed provenance had no significant main treatment or interactive effects on seedling height, leaf number or mycorrhizal colonisation. There were differences in duration of seedling survival between two of the seed provenances: more of the seedlings grown from the Bahill provenance survived than those which originated from the Califer Hill provenance. This effect was most pronounced on the non-sterile treatments of soil 2 and soil 3, and may suggest some degree of variation in edaphic tolerances between those populations. However, provenance differences were not really explored in this experiment: provenances were selected on the basis of seed availability from seed suppliers and germination rates, rather than any systematic examination of habitats.

8.4.6. *Improvements to design of experiment*

More information might have been gained from the experiment if more frequent, non-destructive measures had been taken from the plants. Also, if changes in soil fertility had been monitored throughout the duration of the experiment. The effects of soil factors, which were only assessed for pH and major plant nutrients, as well as changes in soil chemistry induced by the autoclaving process, made some of the results difficult to interpret. It might therefore be useful if future experiments used a range of known symbionts under more standardised substrate conditions.

Lack of familiarity with some of the mycorrhizal techniques precluded a more thorough and frequent assessment of mycorrhizal infection throughout the duration of the experiment. With hindsight, there would also have been some merit in identifying and exploring the species composition of AMF present in each of the soils.

The experiment also indicated that a minimum of two growing seasons were necessary for some of the treatment variables to become apparent, particularly the effects of soil sterilisation. This highlights the importance of monitoring experiments for more than a single growing season.

8.4.7. *Conclusions*

This experiment has reinforced the role of soil type on growth, survival and mycorrhizal colonisation in rowan. This allows us to reject $H_0 2$ that soil factors – an amalgam of physical, chemical and biological characteristics - have no effect on early growth and mycorrhizal infection in rowan. As confirmed by the previous study (Chapter7), rowan does form association with AMF ($H_0 1$), which may benefit growth and survival in marginal conditions. However in more favourable nutrient conditions, indigenous soil micro-organisms may inhibit growth. The experiment also indicated that there were no interaction between host genotype (seed provenance) and AMF ($H_o 5$).

The experiment has some practical implications to nurserymen and landscape practitioners. Soil sterilisation is carried out on many nurseries to control root pathogens, however it also has consequences for soil chemistry and mycorrhizal fungi.

Although heat-sterilisation is no longer widely practised on commercial nurseries in the developed world, restrictions on the use of methyl bromide and other chemicals in the future may change this situation. Non-sterile soil has some potential as a low-cost source of mycorrhizal inoculum, although results may be unpredictable, depending on a range of soil factors, site management, and indigenous micro-organisms (both beneficial and pathogenic), as well as the mycorrhizal dependency of the host plant under consideration. There were differences in the AMF colonisation and growth enhancing properties of the soil samples, which may be attributable both to the chemical properties and also the composition and effectiveness of the indigenous AMF populations present in the soil samples. This latter aspect was not fully explored, but would appear to merit further attention.

CHAPTER NINE

EXPERIMENT TWO: THE EFFECT OF INOCULATION WITH GLOMUS INTRADICES SCHENK & SMITH AND GLOMUS MOSSEAE (NICOL. & GERD.) GERDEMANN & TRAPPE ON GROWTH, AND SEEDLING SURVIVAL IN ROWAN.

9.1. INTRODUCTION

The literature review (Section 3.3) presented evidence that inoculation with AMF improved growth, nutrition and rooting structure in a range of commercially-important woody plant taxa, including apple [78, 73, 74] and cherry[75, 43]. Although few studies used rowan as a host plant, similar results might be expected owing to its close taxonomic relationship to apple and cherry.

Vosatka [115] reported a tentative relationship between root and shoot biomass, and percentage root colonisation in rowan seedlings grown in soils collected from sites affected by sulphur dioxide emissions. The data suggested that seedlings with a higher level of colonisation (>33%) tended to have a greater shoot (X3) and root (X3.5) dry mass than those associated with a lower level of AMF colonisation (4%). However, soils varied in nutrient status, site history and vegetation cover, as well as numbers of infective propagules, and so his results need to be interpreted with caution. Morrison *et al.* [80] inoculated a range of woody plants including rowan, green ash (*Fraxinus pennsylvanica*) and apple (*Malus* spp. with *Glomus fasiculatum* and *G. intradices* applied as a root dip prior to planting. Inoculation increased percentage root colonisation but had no effects on growth. However when the trees were later transplanted to a second field site, stem diameter (for rowan only) was significantly greater after two years in trees that had previously been inoculated with *G. intradices* (X1.2) compared with inoculation with *G. fasciculatum* or the non-inoculated controls. This data also suggests preferential host-endophyte interactions.

The phenomena of host-endophyte interactions has also been observed by other studies. Benson and Covey [242] found that *G. fasiculatum* was more effective in promoting growth in apple seedlings (X 2.8) than *G. mosseae* (X 2.5) compared to non-inoculated plants. Gardiner and Christenson [83] observed differences in growth between pear seedling inoculated with *G. intradices* and *G. deserticola* related to soil P levels: at low levels (0.3 to 0.25 mg $^{-1}$ l P) greatest growth was observed with *G. intradices*; at high levels (0.40 mg $^{-1}$ l P) greatest growth was associated with *G. deserticola*.

Interactions have also been reported between endophyte and host genotype. Morin *et al.*[74] inoculated a range of apple rootstocks and observed differences in height, leaf surface area and percentage colonisation between the rootstock cultivars. In general, the M26 rootstock responded better to inoculation than the P16 rootstock. They also observed that of the range of AM isolates used, an isolate of *G. versiforme* from California performed better in terms of improved nutrition, shoot height and dry weight compared to non-inoculated control plants. This was despite taking slightly longer following inoculation (8-10 weeks) for effects to become significant than the other AM isolates (6 weeks). They attributed the greater efficiency of this isolate to its more extensive hyphal network rather than percentage root colonisation.

The previous experiment (Chapter 8) suggested a relationship between soil micro-organisms such as AMF, and growth in rowan. However the effects were strongly mediated by soil factors such as initial soil nutrient status, as well as a range of other factors including AMF populations which were not assessed. This made some of the results difficult to interpret. A second experiment, using known AMF isolates, under

less variable substrate and nutrient status was therefore proposed to consider the following null hypotheses:

H_o3. Inoculation from AMF has no effect on the early growth of rowan.

H_o4. All AMF species are equally effective endophytes of rowan.

H_o5. There are no interactions between AMF and host genotype (seed provenance).

It was also anticipated that the experiment would provide inoculated plant material that could be used in a later field experiment.

9.2. MATERIALS AND METHOD

Seed material

In December 1994, several Scottish seed provenances of *Sorbus aucuparia* L., were obtained from the seed company 'Forestart' . The seeds were stratified for 3 months at 1-2^{o}C, prior to sowing in vermiculite in March 1995. Only seeds from Loch Naver (NC 6136) and Pluscarden (NJ 1455) produced sufficient seedlings for the level of replication required by the experiment.

Substrate preparation

In July 1995, the seedlings were potted into 0.3 l pots filled with dried silica sand (grade 16/30) obtained from Levenseat Quarry, Fauldhouse, West Lothian. The sand had been autoclaved twice for 60 minutes at 121 ^{0}C to destroy any contaminating organisms, prior to thorough mixing with the mycorrhizal inoculum. Inoculum of *Glomus intradices* Schenk & Smith (GI) and *G. mosseae* (Nicol. & Gerd.) Gerdemann & Trappe, (GM) was obtained from MicroBio Limited, as an 8/16 mesh size formulation on clay particles. Two levels of inoculum were incorporated with the sand: a low dose

of 5g l^{-1} and a high dose of 25g l^{-1}.

Experimental Design

The pots were arranged in two plots (containing all of the treatments) on the same glasshouse bench. There were 25 plants for each of the endophyte and provenance treatments. This is indicated below in Figure 9.1. Greater randomisation of treatments was not possible because of the need to avoid cross-contamination between treatments.

Figure 9.1. Experimental Design for Experiment Two.

Glasshouse wall (Plot One)										
Non-inoculated Controls		*Glomus mosseae*				*Glomus intradices*				
No Endophyte		Low [a]		High [b]		Low		High		
L[c]	P	L	P	L	P	L	P	L	P	
Glasshouse path										

Glasshouse wall (Plot Two)										
Glomus intradices				Non-inoculated controls		*Glomus mosseae*				
High		Low		No endophyte		High		Low		
P	L	P	L	P	L	P	L	P	L	
Glasshouse path										

[a] Low inoculation dosage : 1.5 g per 0.3 l pot
[b] High inoculation dosage : 5.5 g per 0.3 l pot
[c] Seed provenance : L = Loch Naver; P = Pluscarden

Liquid Feed

The sand cultures were fed twice weekly with half strength Hoaglands solution with added micronutrients; which was stored in a refrigerator until required (Table 9.1.)

Table 9.1. Details of nutrients in half-strength Hoaglands liquid feed

Hoaglands Stock Solution mixed with 10 litres distilled water (half – strength)	A-Z Micro-nutrient solution Mixed with 200 ml distilled water
2.55 g KNO_3 4.1 g $Ca(NO_3)_2$ 2.45 g $MgSO_4.7H_2O$ 0.68 g KH_2PO_4 5 ml ferric tartrate 0.5% 5 ml A-Z micro-nutrients	0.572 g H_3BO_3 0.362 g $MnCl_2.4H_2O$ 0.044 g $ZnSO_4.7H_2O$ 0.176 g $CuSO_4.5H_2O$ 0.005 g $H_2MoO_4.H_2O$

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Assessments

Seedling height and leaf number were assessed at four week intervals during the first growing season. In September 1995 (11 weeks after inoculation) and June 1996 (46 weeks after inoculation) the following assessments were made:

1. Plant height (in mm)
2. Condition (a subjective scale of 0-4 where 0 = dead, 1= stunted and chlorotic, 2 = chlorotic, 3 = chlorotic but recovering, 4 = healthy)
3. Leaf number
4. Seedling survival.

In September 1995, a randomly selected sample of 5 plants per treatment was harvested. The shoots and roots were separated and fresh weights determined. Shoot dry weights were obtained by heating in an oven at 70 ^{0} C until no further changes in weight were recorded, and the roots stained and assessed for percentage mycorrhizal colonisation (Sections 6.3 and 6.4).

In 1996 due to the high level of seedling mortality, all plants (n=25) were harvested at the end of June, and assessed for fresh and dry weight, and percentage mycorrhizal infection.

9.3. RESULTS

9.3.1. Statistical analysis

As in the first experiment, there were problems with skewed distributions and missing values due to seedling mortality. This meant that many of the statistical procedures which assume normal data distributions were inappropriate, and so it was decided to use Multiple Linear Regression Analysis (MLRA) to assess the overall influence of all the treatment factors used in the experiment. An explanation of the treatment factor levels in given below in Table 9.2.

Table 9.2. Treatment factor levels and dependent variables.

Treatment Factor Levels

- Endophyte: 3 levels (No endophyte; *Glomus intradices*; *G. mosseae*)
- Dosage: 2 levels (low dosage; high dosage)
- Provenance: 2 levels (Loch Naver; Pluscarden)

Dependent Variables (1995 and 1996)

- Height (in mm)
- Seedling Condition
- Leaf number (mean per plant)
- Shoot dry weight (g)
- Root fresh weight (g)
- Percentage mycorrhizal colonisation

The resulting accumulated analysis of variance derived from MLRA across all the dependent variables in 1995 and 1996 is given in Table 9.3.

Table 9.3. Overall accumulated analysis of variance (F-Statistic, degrees of freedom, significance.

	Height 1995	**Condition 1995**	**Leaf number 1995**	**Shoot dry weight 1995**	**Root fresh weight 1995**	**Percentage mycorrhizal colonisation 1995**
Main Effects						
Provenance	**52.99, 1** ***	2.69, 1 ns	**9.16, 1,** ***	1.42, 1, ns	2.47, 1, ns	3.29, 1, ns
Endophyte	**12.42, 2** ***	**8.52, 2,** ***	**9.40, 1,** ***	**3.32, 2,** *	2.55, 2, ns	**46.65, 2,** ***
Dosage	0.59, 1 ns	0.6, 1, ns	0.13, 1, ns	0.81, 1, ns	2.17, 1, ns	**60.49, 1,** ***
2-way interactions						
Provenance * Endophyte	2.58, 2 ns	**4.53, 2** *	**4.46, 2,** *	0.89, 2, ns	0.58, 2, ns	1.41, 2, ns
Provenance * Dosage	0.01, 1, ns	1.25, 1, ns	**5.34, 1,** *	0.14, 1, ns	1.01, 1, ns	2.40, 1, ns
Endophyte * Dosage	**9.30, 1,** **	1.22, 1, ns	0.38,1, ns	0.78, 1, ns	1.45, 1, ns	**63.96, 1,** ***
3-way interactions						
Provenance * Endophyte * Dosage	1.21, 1, ns	**5.68, 1,** *	3.12, 1, ns	0.00, 1, ns	0.00, 1, ns	1.28, 1, ns
	Height 1996	**Condition 1996**	**Leaf number 1996**	**Shoot dry weight 1996**	**Root fresh weight 1996**	**Percentage mycorrhizal colonisation 1996**
Main Effects						
Provenance	0.00, 1, ns	0.02, 1, ns	1.04, 1, ns	0.25, 1, ns	1.77, 1, ns	0.49, 1, ns
Endophyte	**4.62, 2** *	0.91, 2, ns	0.88, 2, ns	0.46, 2, ns	**4.93, 2,** *	**7.86, 2,** **
Dosage	0.36, 1, ns	1.16, 1, ns	0.04, 1, ns	0.23, 1, ns	0.20, 1, ns	0.77, 1, ns
2-way interactions						
Provenance * Endophyte	0.37, 1, ns	0.00, 1, ns	0.07, 1, ns	0.07, 1, ns	0.02, 1, ns	0.01, 1, ns
Provenance * Dosage	0.00, 1, ns	0.23, 1, ns	0.00, 1, ns	0.04, 1, ns	0.19, 1, ns	2.52, 1, ns
Endophyte * Dosage	0.91, 1, ns	0.77, 1, ns	0.41, 1, ns	0.14, 1, ns	0.17, 1, ns	0.00, 1, ns

In 1995 there were a number of interaction and main treatment effects; in 1996 only endophyte was significant as a main treatment effect. In the following sections, the results for the dependent variables will be considered individually, dealing with significant treatment interactions, followed where appropriate by main treatment effects.

9.3.2. Seedling Height.

Seedling height was measured at the end of 1995 and 1996, as well as at four-week intervals during 1995. The end of year data will be considered first of all. At the end of 1995 there was a significant interaction between endophyte and inoculum dosage ($P<0.01$). The tallest seedlings were those inoculated with the high dosage of GI (27 mm) followed by the low dosage of GI (22 mm), and the non-inoculated controls (22 mm). Seedlings inoculated with either dosage of GM were the smallest: 17 mm (GM high dosage), 20 mm (GM low dosage). There was also a very highly significant ($P<0.001$) main treatment effect for seed provenance: Pluscarden seedlings were taller (26 mm) than those from Loch Naver (18 mm).

By 1996 only endophyte was significant as a main treatment effect ($P<0.05$); there were no significant treatment interaction. Seedlings inoculated with GI were twice as tall (145 mm) as the non-inoculated seedlings (79 mm) or those inoculated with GM (72 mm). The effects of the endophyte treatments in 1995 and 1996 are shown below in Table 9.4 and Figure 9.2.

Figure 9.2. The effect of endophyte treatment on seedling height in 1995 and 1996.
(Signficant differences apply within year only)

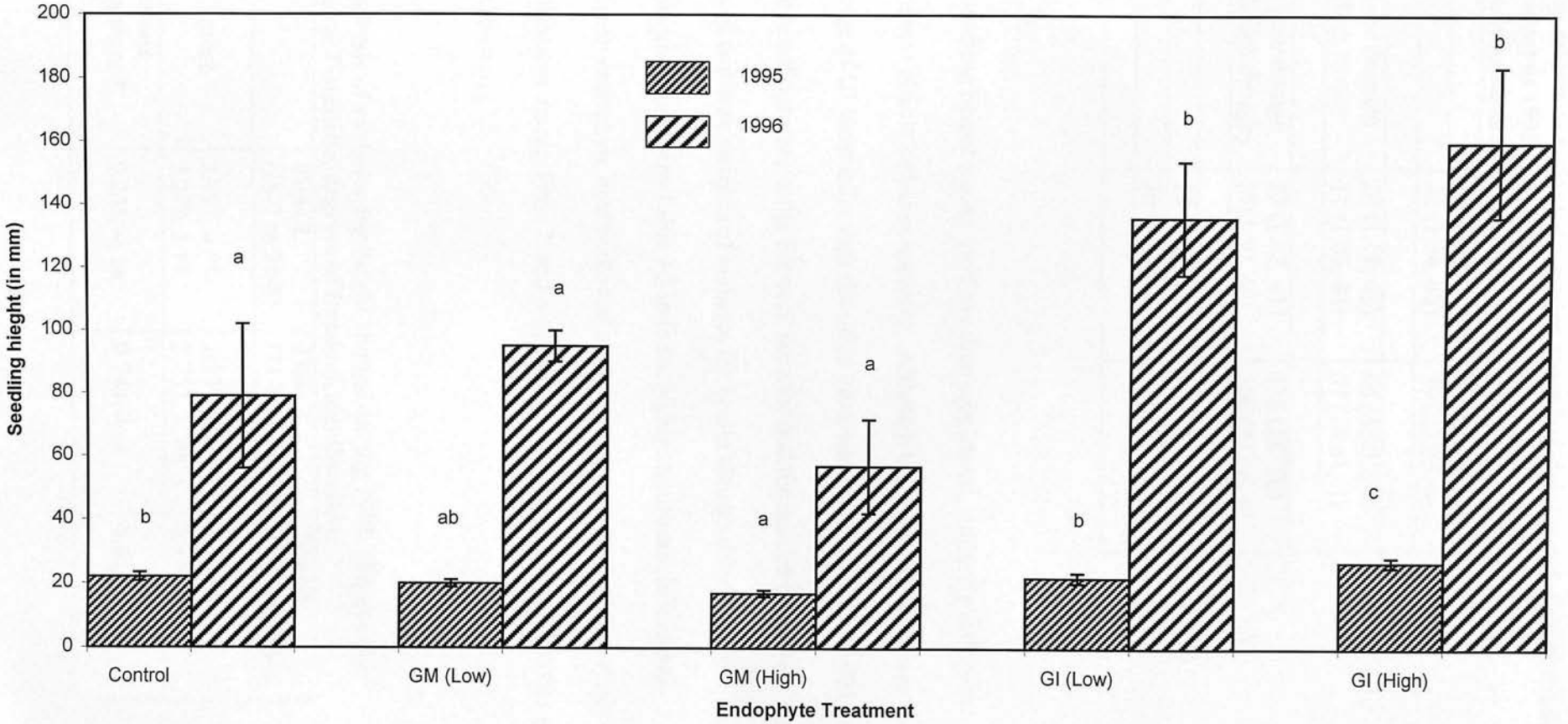


Table 9.4. The effects of endophyte species, inoculum dosage and seed provenance on height (in mm) in 1995 and 1996.
Figures in brackets indicate standard error of mean and sample size (n).

Factor	Height in 1995	Height in 1996
Control	22 (1.34, 48)	79 (22.95, 4)
G. mosseae (Low dosage)	20 (1.18, 45)	95 (5.00, 2)
G. mosseae (High dosage	17 (1.05, 44)	57 (14.81, 3)
G. intradices (Low dosage	22 (1.63, 45)	136 (18, 10)
G. intradices (High dosage)	27(1.50, 46)	160 (23.66, 6)
Loch Naver	18 (.074, 112)	121 (23.79, 5)
Pluscarden	26 (0.89, 116)	120 (14.13, 20)

If changes in seedling height during 1995 are then considered, a slightly different pattern of treatment effects becomes apparent. Although the seedlings inoculated with the higher dosage of GI were taller than the other treatments at baseline ($P<0.001$), the non-inoculated seedlings grew more between baseline and the second measure 4 weeks later. A series of two-way analysis of variance for height change were therefore carried out. The results given below in Table 9.5 indicate highly significant differences ($P<0.001$) between endophyte treatments and seed provenances during Time 1; no significant differences during Time 2, and a significant endophyte treatment difference during Time 3 ($P<0.01$).

Table 9.5. Analysis of variance for height change during 1995. (Figures in brackets indicate F-statistic, degrees of freedom, significance).

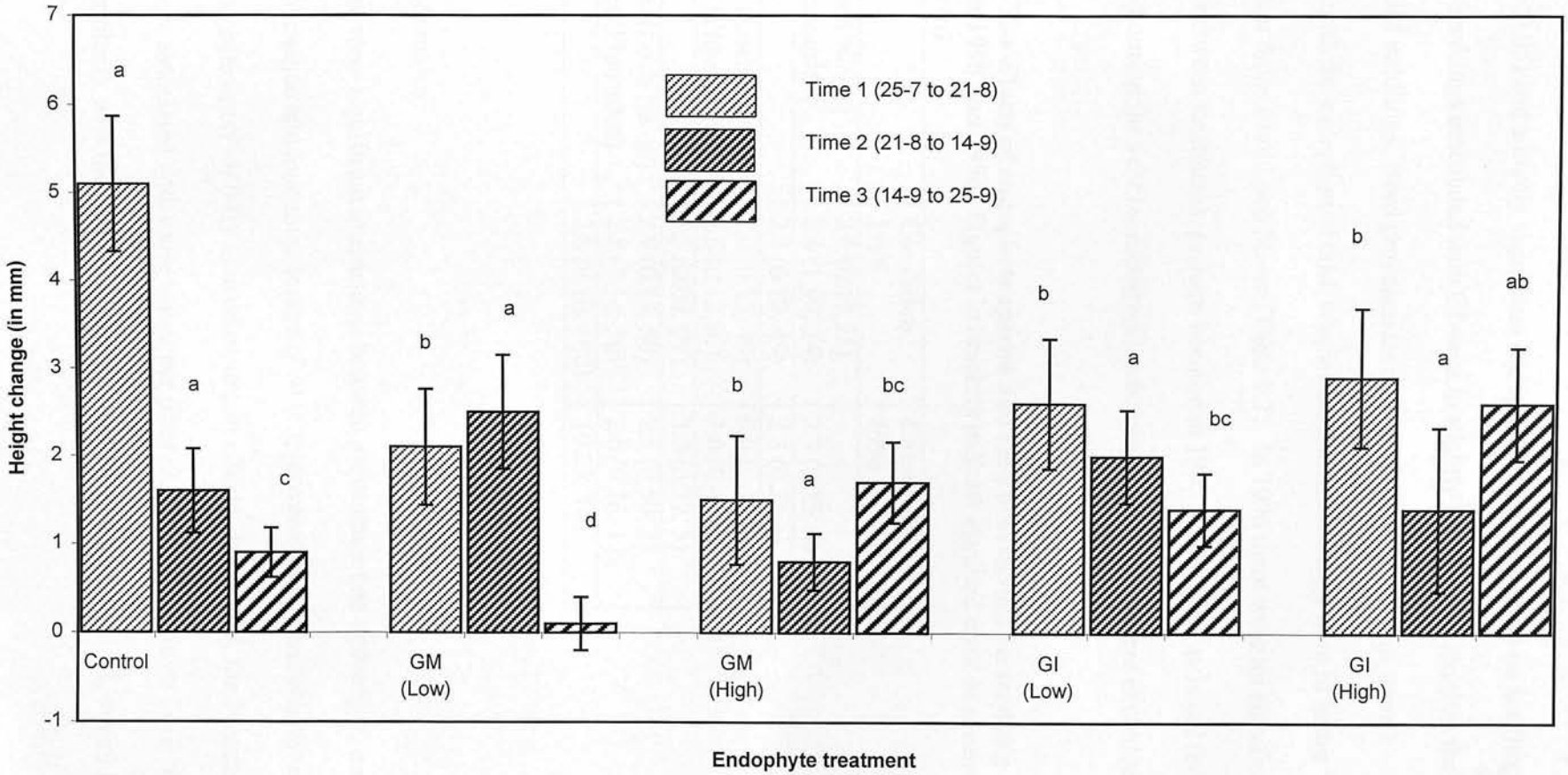
	Time 1 (25-7 to 21-8)	Time 2 (21-8 to 14-9)	Time 3 (14-9 to 25-9)
Main Effects			
Endophyte treatment	**3.497, 4 ****	1.137, 4 ns	**4.507, 4 ****
Provenance	**7.273, 1 ****	2.751, 1, ns	0.776, 4 ns
2-way interactions			
Endophyte treatment * Provenance	0.243, 4, ns	0.744, 4 ns	0.612, 4 ns

Height changes for these time periods are given below in Table 9.6, and indicate that during the first time period the non-inoculated control seedlings grew nearly twice as fast as any of the inoculated seedlings: 5 mm in 4 weeks compared to 2-3mm in the inoculated seedlings. During the second time period, there were no differences in growth rate between the treatments. However by the third time period, the higher dosage of GI were growing at the fastest rate (2.6 mm in 2 weeks), followed by the higher dosage of GM (1.7 mm), the lower dosage of GI (1.4 mm) and the controls (0.9 mm). The lower dosage of GM were growing significantly slower (0.1 mm) than any of the other treatments. These results would appear to suggest that the inoculated plants experienced an initial growth depression during the first time period, but that by the third time period were on the whole growing faster than the non-inoculated plants. This is indicated in Figure 9.3.

Table 9.6. Seedling height change (in mm) between time periods in 1995 for endophyte treatment and seed provenance.
Figures in brackets indicate standard error and sample size (n); treatment means followed by different lower case letter, differ at P<0.05 using Least Significant differences.

Factor	**Time 1 (25-7 to 21-8)**	**Time 2 (21-8 to 14-9)**	**Time 3 (14-9 to 25-9)**
Control	5.1 a (0.77,48)	1.6 (0.48, 47)	0.9 c (0.28, 48)
G. mosseae (Low dosage)	2.1 b (0.66, 47)	2.5 (0.65,46)	0.1 d (0.30, 45)
G. mosseae (High dosage	1.5 b (0.73, 46)	0.8 (0.32, 44)	1.7 bc (0.46, 44)
G. intradices (Low dosage	2.6 b (0.74, 47)	2.2 (0.53, 43)	1.4 bc (0.41, 45)
G. intradices (High dosage)	2.9 b (0.79, 47)	1.4 (0.93, 44)	2.6 ab (0.64, 46)
Loch Naver	2.0 (0.46, 117)	1.2 (0.4, 111)	1.2 (0.31, 112)
Pluscarden	3.7 0.48, 118)	2.2 (0.38, 113)	1.5 (0.25, 116)

Figure 9.3. The effect of endophyte treatment on changes in seedling height by time period.



9.3.3. Seedling condition.

In 1995 there was a highly significant interaction between provenance and endophyte on seedling ($P<0.05$) and a highly significant endophyte effect ($P<0.001$) on seedling condition. Seedlings inoculated with GI were in slightly better condition than the control or GM seedlings. Seed provenances were more or less the same across endophyte, with the exception of GM, where Pluscarden seedlings were in better condition than those from Loch Naver (Table 9.7). In 1996 there were no significant differences between treatments, perhaps because in 1995 'condition' included those that had died during the year (condition=0), whereas in 1996, these were excluded.

Table 9.7. The effects of endophyte species and seed provenance on seedling condition in 1995 and 1996. Figures in brackets indicate standard error of mean and sample size (n)

Factor	Condition 1995	Condition 1996
Control (Loch Naver)	2.6 (0.19,25)	-
Control (Pluscarden)	2.4 (1.90, 24)	2.3 (0.25, 4)
Mean	2.5 (0.12, 49)	2.3 (0.25,4)
G. mosseae (Loch Naver)	2.0 (0.15, 49)	3.0 (-, 1)
G. mosseae (Pluscarden)	2.6 (0.12,50)	3.0 (0.41, 4)
Mean	2.3 (0.09, 99)	3.0 (0.32, 5)
G. intradices (Loch Naver)	2.9 (0.13, 50)	2.5 (0.50, 2)
G. intradices (Plucarden)	2.8 (0.14, 50)	2.6 (0.26, 12)
Mean	2.8 (0.10, 100)	(0.23, 14)

9.3.4. *Leaf number.*

In 1995 there were significant interactions between provenance and endophyte, and between provenance and inoculum dosage ($P<0.05$); provenance and endophyte were also highly significant ($P<0.001$) as main treatment effects. In general, the Pluscarden seedlings were associated with more leaves per plant (3.5) than those from Loch Naver (4 leaves per plant), with the exception of the high inoculum dosage of GI, where Loch

Naver seedlings had slightly more leaves (4.5) than Pluscarden (3.9). In 1996, there were no significant treatment effects on leaf number (Table 9.8).

Table 9.8. The effects of seed provenance, endophyte species and inoculum dosage on leaf number in 1995 and 1996.
Figures in brackets indicate standard error of mean and sample size (n).

Factor	Leaf number 1995	Leaf number 1996
1. Control		
Loch Naver	3.9 (0.29, 24)	-
Pluscarden	3.9 (0.34, 24)	9.3 (3.25, 4)
Mean	3.9 (0.22, 48)	9.3 (3.25, 4)
2. G. mosseae (Low)		
Loch Naver	2.7 (0.21, 21)	-
Pluscarden	3.9 (0.19, 22)	10.5 (0.50, 2)
3. G. mosseae (High)		
Loch Naver	2.7 (0.30, 21)	10.0 (-, 1)
Pluscarden	3.8 (0.19, 22)	14.0 (2.00, 2)
Mean	3.3 (0.13, 88)	11.8 (1.11, 5)
4. G. intradices (Low)		
Loch Naver	3.5 (0.30, 22)	9.0 (-, 1)
Pluscarden	4.5 (0.29, 23)	13.8 (2.13, 9)
5. G. intradices (High)		
Loch Naver	4.5 (0.22, 23)	9.7 (2.67, 3)
Pluscarden	3.9 (0.27, 23)	13.0 (2.08, 3)
Mean	4.1 (0.14, 91)	12.6 (1.36, 16)
Loch Naver	3.5 (0.13, 111)	9.6 (1.47, 5)
Pluscarden	4.0 (0.12, 116)	12.5 (1.21, 20)

9.3.5. Shoot dry weight

In 1995, only endophyte species had a significant effect on shoot dry weight ($P<0.05$): seedlings inoculated with GI had a greater shoot dry weight (0.08 g) than either non-inoculated (0.06 g) or GM inoculated seedlings (0.04 g). Although not statistically significant, Pluscarden seedlings had a slightly higher dry weight (0.07 g) than Loch Naver seedlings (0.05 g). In 1996, there were no significant treatment effects (Table 9. 9 and Figure 9. 4)

Figure 9.4. The effect of endophyte treatment on shoot dry weight in 1995 and 1996.
(Significant differences apply within year only)

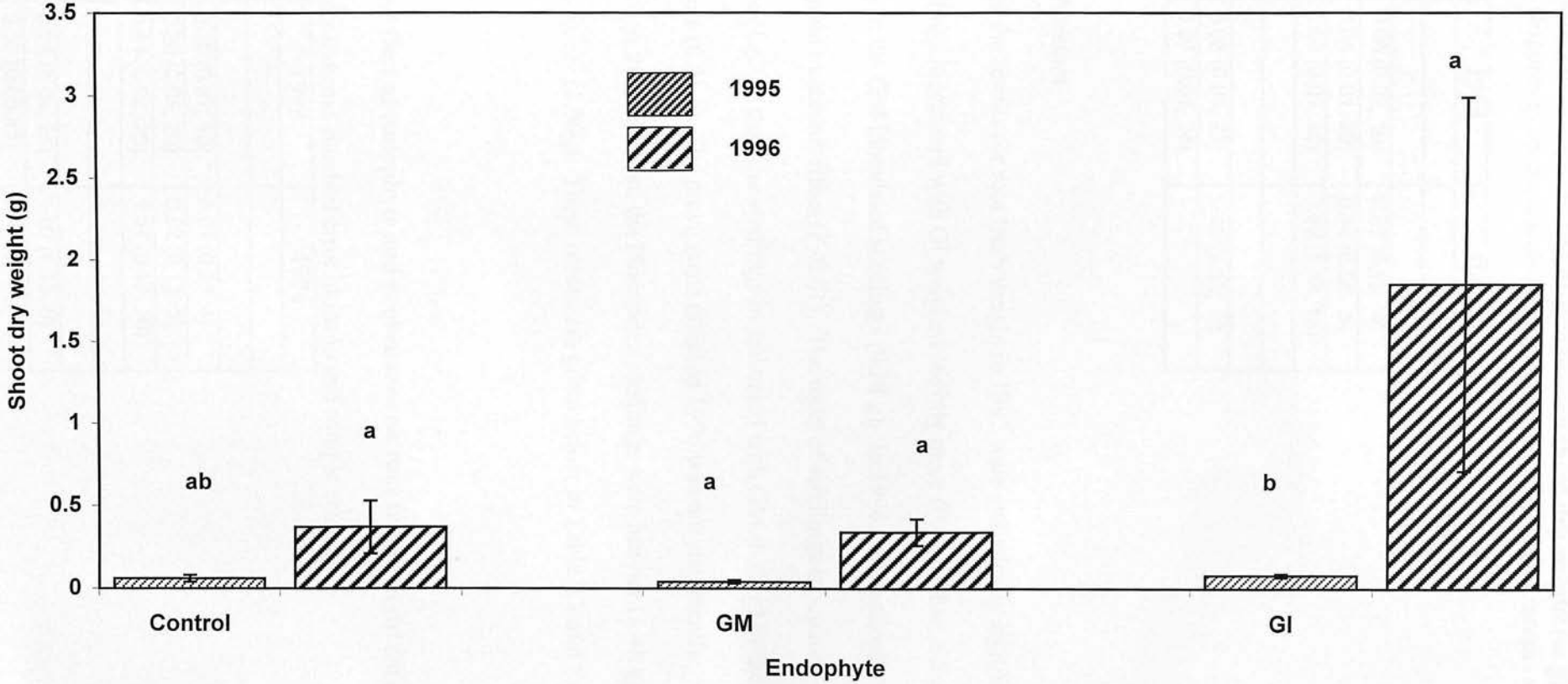


Table 9.9. The effect of endophyte and provenance on shoot dry weight (in g) in 1995 and 1996. (Figures in brackets indicate standard error of mean and sample size (n).

	1995	1996
Endophyte:		
Control	0.06 (0.02, 10)	0.37 (0.16, 4)
G. mosseae	0.04 (0.01, 20)	0.34 (0.08, 5)
G. intradices	0.08 (0.01, 20)	1.85 (1.14, 16)
Provenance:		
Loch Naver	0.05 (0.01, 25)	0.48 (0.10, 5)
Pluscarden	0.07 (0.01, 25)	-

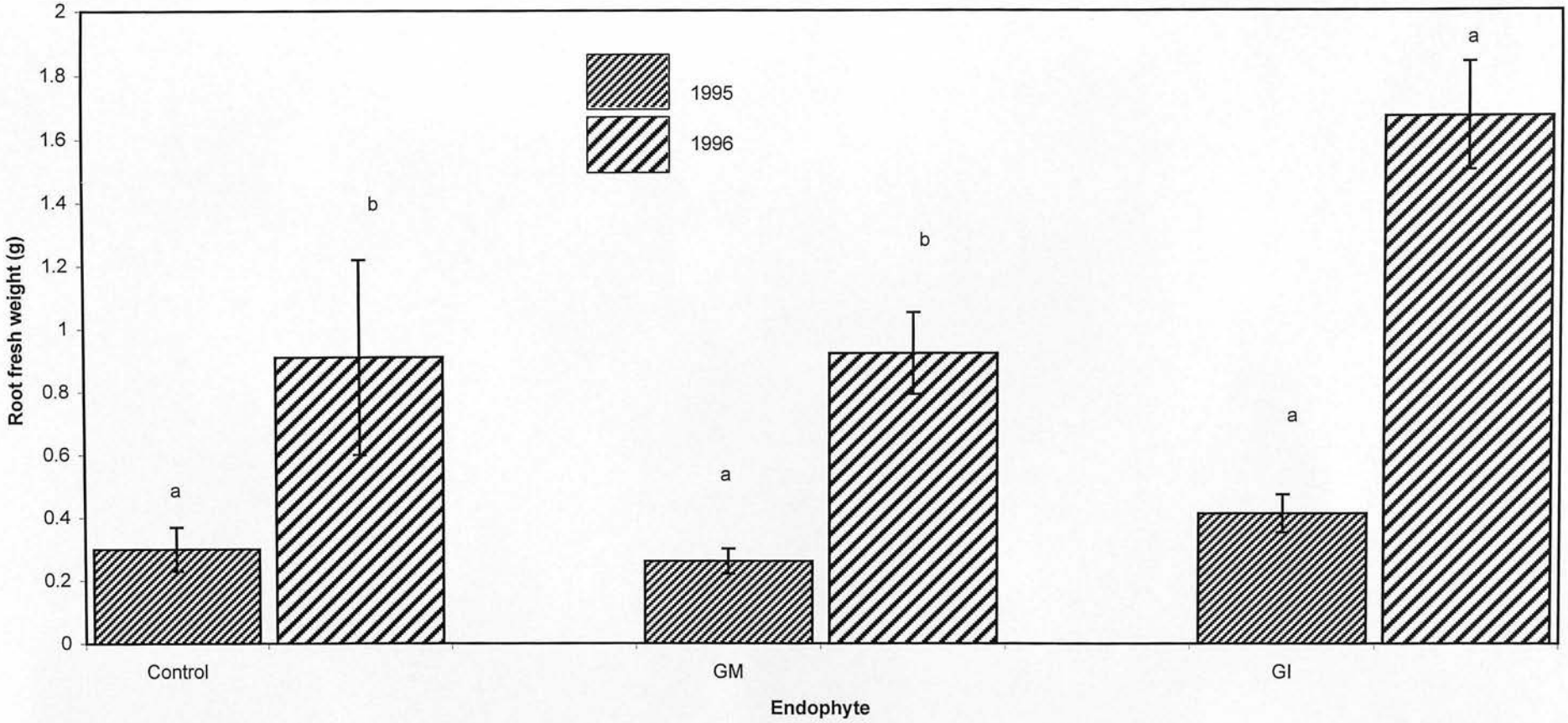
9.3.6. Root fresh weight

Although none of the results for root fresh weight in 1995 were statistically significant, the roots of seedlings inoculated with GI weighed slightly more (0.41 g) than either the controls (0.30 g) or the GM inoculated seedlings (0.28 g). In 1996, only endophyte was significant as a main treatment effect ($P<0.01$). The roots of seedlings inoculated with GI weighed more (1.67 g) than the seedlings inoculatated with GM (0.92 g) or the non-inoculated controls (0.91 g). The provenance effect in 1996 was not statistically significant, although the roots from the Pluscarden seedlings were heavier (1.48 g) than those from Loch Naver (1.06g). These results are given below in Table 9.8 and Figures 9.5.

Table 9.10. The effect of endophyte and provenance on root fresh weight (in g) in 1995 and 1996.
Figures in brackets indicate standard error of mean and sample size (n).

	1995	1996
Endophyte:		
Control	0.30 (0.07, 10)	0.91 (0.31, 4)
G. mosseae	0.26 (0.04, 20)	0.92 (0.13, 5)
G. intradices	0.41 (0.06, 20)	1.67 (0.17, 16)
Provenance:		
Loch Naver	0.28 (0.04, 25)	1.06 (0.15, 5)
Pluscarden	0.37 (0.05,25)	1.48 (0.17, 20)

Figure 9.5. The effect on endophyte treatment on root fresh weight in 1995 and 1996.
(Significant differences apply within year only)



9.3.7. Percentage mycorrhizal colonisation

In 1995, there was a highly significant interaction between endophyte and inoculum dosage ($P<0.001$). The high dosage of the GI inoculum was nearly ten times more effective at colonising the rowan roots (53%) compared to the low dosage of GI (6%), or either of the GM dosages (4%). It is interesting to note that the low dosage of GI was not significantly better at colonising the roots than any of the GM treatments.

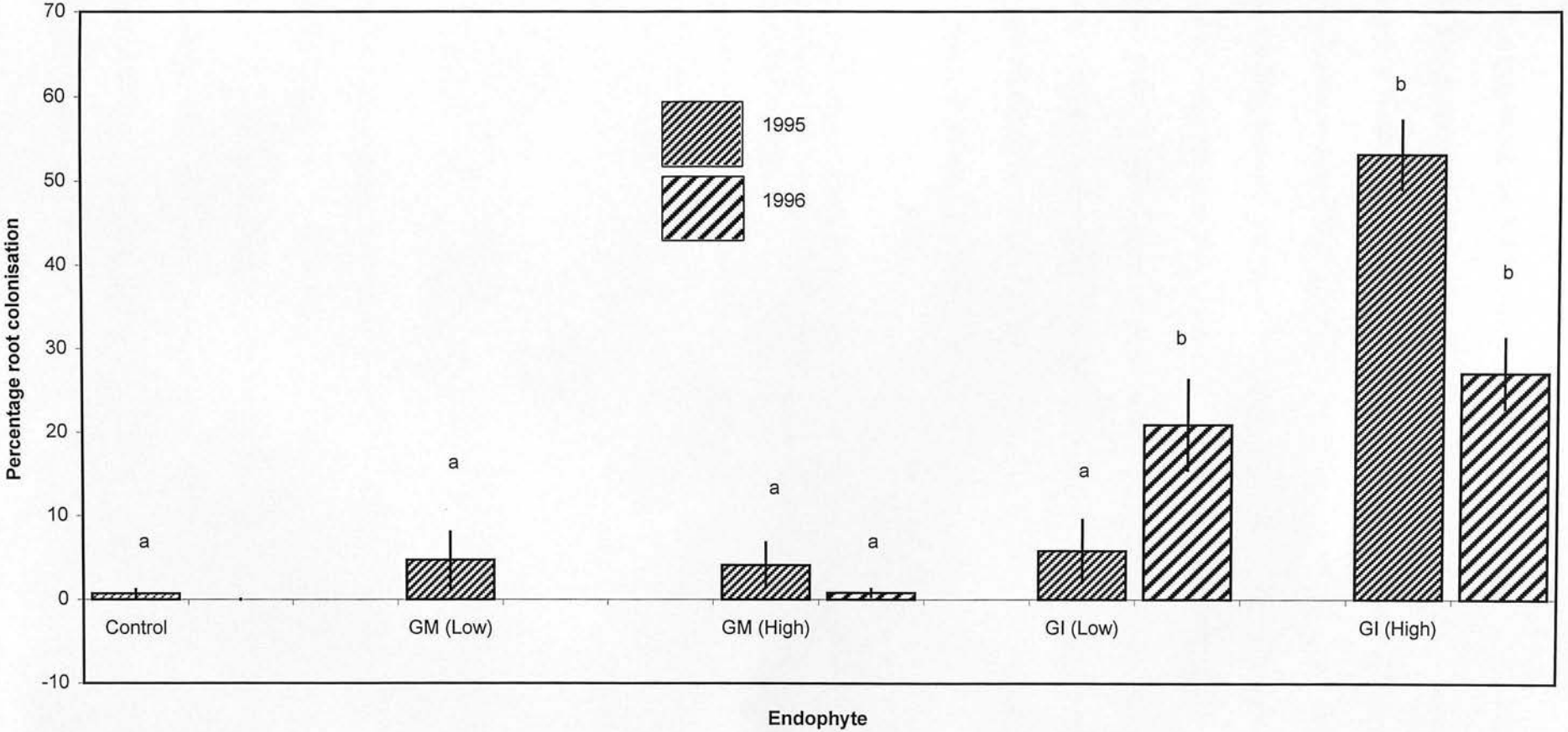
In 1996 only endophyte was significant as a main treatment effect ($P<0.01$). The GI inoculated seedlings had 23% root colonisation; the GM treatments less than 1%. This would appear to indicate that only the GI propagules survived the winter and re-established root infections. These results are given below in Table 9.11 and Figures 9.6.

Table 9.11. The effect of endophyte, inoculum level and provenance on percentage mycorrhizal infection in 1995 and 1996.
Figures in brackets indicate standard error of mean and sample size (n).

Endophyte	Level	Percentage colonisation in 1995	Percentage colonisation in 1996
Control	**None**	0.7 a[i] (0.49, 10)	0.0 a (0.04, 4)
G. mosseae	**Low**	4.7 a (3.37, 10)	0.0 a (-, 1)
	High	4.1 a (2.69, 10)	0.8 a (0.47, 3)
	Mean	4.1 (2.10, 20)	0.6 (0.38, 4)
G. intradices	**Low**	5.8 a (3.74, 10)	20.9 b(5.42,10)
	High	53.1 b (4.11, 10)	27.0 b(4.23, 6)
	Mean	29.5 (6.06,20)	23.2 (3.72,16)
Provenance:			
Lochnaver		11.3 (4.02, 25)	19.2 (7.82, 5)
Pluscarden		16.1 (4.48, 25)	14.6 (3.74, 19)

[i] Means within year, followed by same lower case letter do not differ significantly using LSD at $P<0.05$

Figure 9.6. The effect of endophyte on percentage mycorrhizal colonisation.
(Significant differences apply within year only)



9.3.8. Seedling survival.

Winter 1995-6 was extremely cold with temperatures below –20 ^{0}C for over a week in Edinburgh. Despite being in a heated (frost-protection) glasshouse, the pots became frozen, and many seedlings died: only 25 out of the 178 seedlings alive at the end of 1995 were still alive in June 1996. In order to assess the contribution of the treatment variables to seedling survival, a series of chi-square tests were calculated. Survival was classified as : 1 = survived less than 1 year; 2 = survived one year, and 3 = survived more than one year (i.e. alive at end of experiment). The results of the chi-square tests are shown in Table 9.12 and indicate that only endophyte ($P<0.05$) and provenance ($P<0.01$) were significant as main treatment effects. There were no significant treatment interaction effects on survival (Table 9.12).

Table 9.12. Chi-square tests of seedling survival : the contribution of endophyte species, inoculum level and seed provenance.

Source of Variation	Chi-square	Degree of Freedom	Significance
Endophyte	6.40	2	* [1]
Endophyte * Level	0.11	4	ns
Endophyte * Provenance	1.04	2	ns
Endophyte * (Provenance * Level)	1.47	4	ns
Provenance	10.32	1	**
Provenance * Level	3.49	2	ns
Provenance * Endophyte	2.53	2	ns
Provenance * (Endophyte * Level)	0.38	4	ns

[1] * = significant at $P<0.05$; ** = highly significant at $P<0.01$; ns = not significant.

During the first year, slightly more of the seedlings inoculated with GM (11%) died than either the GI treatment (9 %) or the non-inoculated controls (4 %). If provenance is considered, more Loch Naver seedlings died during the first year (10%) than Pluscarden (7 %). However, by the start of 1996, huge losses had occurred across all treatments (after adjusting for the 50 seedlings destructively sampled in 1995) of the 178 seedlings alive at the end of 1995, only 25 were still alive in June 1996 (Table 9.13 and Figure 7).

Figure 9.7. The effect of endophyte treatment on seedling survival in 1995 and 1996

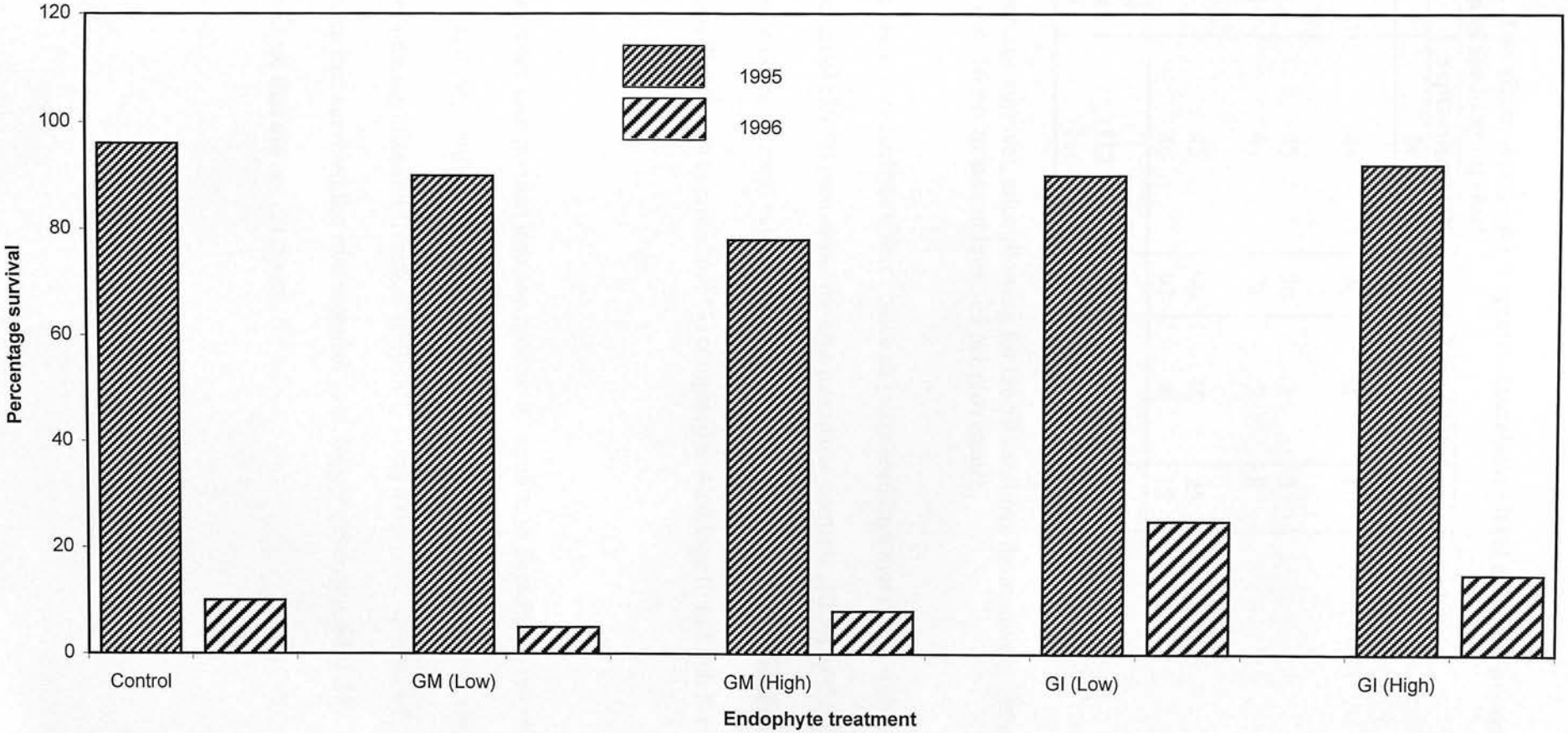


Table 9.13. The effect of endophyte species, inoculation level and seed provenance on duration of seedling survival.

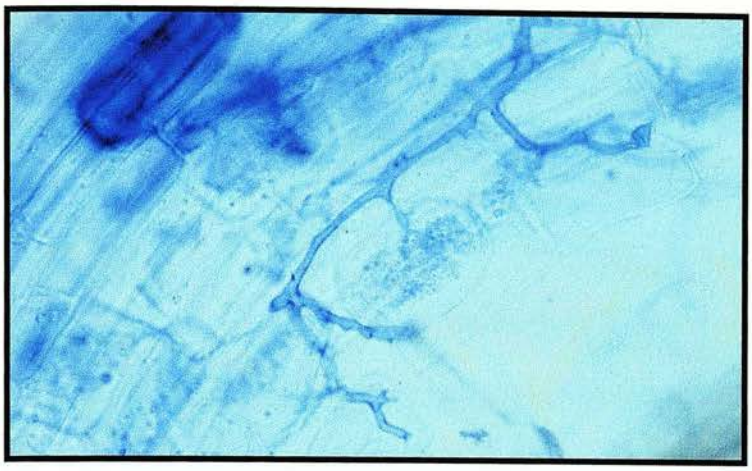
	September 1995		June 1996	
	N	%	N	%[a]
Treatment				
Control	48	96	4	10
GM (Low)	45	90	2	5
GM (High)	44	78	3	8
GI (Low)	45	90	10	25
GI (High)	46	92	6	15
Provenance				
Loch Naver	112	90	5	10
Pluscarden	116	93	20	40

[a] Total percentage survival, after allowing for the 50 seedlings destructively sampled at the end of 1996: 10 per treatment level; 25 per provenance.

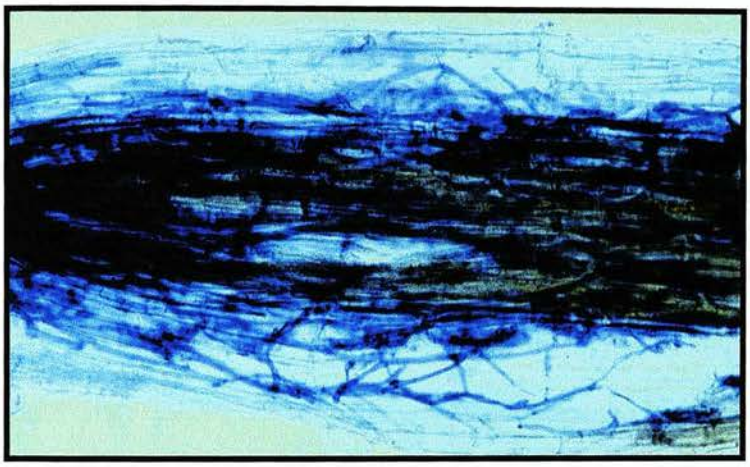
There was a marked endophyte effect : twice as many seedlings inoculated with *G. intradices* survived (20 %) than either the non-inoculated controls (10 %) or those inoculated with *G. mosseae* (6 %). Provenance was also significant: four-times as many seedlings from Pluscarden survived (40 %) compared to seedlings from Loch Naver (10 %).

The seedlings were then divided into two groups: those alive in September 1995 but not in June 1996, and those still alive in June 1996, and compared by height at the end of 1995. There were significant differences ($P<0.001$) using a one-way analysis of variance. Those that survived the winter tended to be bigger (29.6 mm, SE 1.58) compared to those that did not (21.5 mm, SE 0.78).

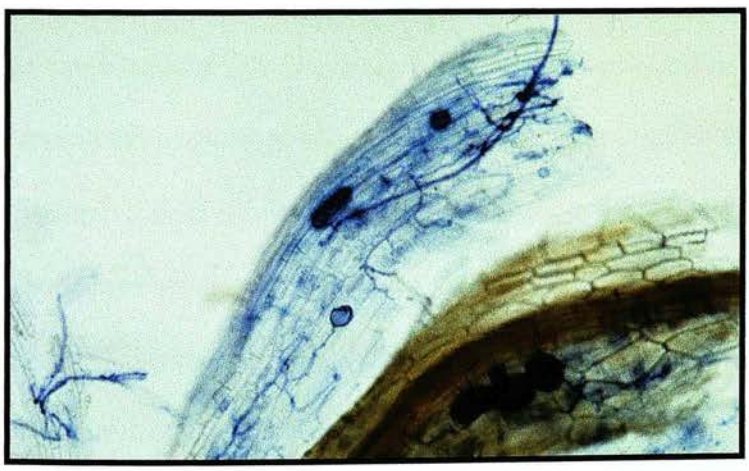
Figure 9.8 Root colonisation in rowan following inoculation



G. intradices (x 400)



G. mosseae (x200)



G. intradices (x100)

9.4. DISCUSSION

9.4.1. Introduction

The aims of this experiment were to consider the effects of inoculation with *Glomus intradices* (GI) and *G. mosseae* (GM) on seedling growth and survival in rowan, and also to consider whether there were any interactive effects between AMF and seed provenance. A factorial experiment was therefore set up comprising of endophyte species (3 levels), inoculum dosage (3 levels) and seed provenance (2 levels). A preliminary literature search indicated that AMF improve growth and survival in woody plants, through improvements to host nutrition, rooting structure and physiology.

9.4.2. Summary of treatment effects

1. Inoculation with AMF improved the growth and survival of rowan seedlings.
2. There were differences in the ability of the two endophyte to infect roots, and promote growth across the measured parameters: GI was more infective and effective than GM; GM inhibited growth in comparison to non-inoculated seedlings.
3. A higher dosage of inoculum of GI increased beneficial effects; in GM a higher dosage tended to reduce beneficial effects.
4. Inoculation decreased the rate of growth in inoculated compared to non-inoculated seedlings during the early stage of growth, however by the end of the season, inoculated plants of both endophyte species appeared to be growing at a faster rate than non-inoculated seedlings.
5. Inoculation with GI improved the ability of rowan to survive severe environmental stress (freezing and drought), although this may have been attributable to the larger size of the GI seedlings.
6. Provenance had an effect on seedling height and leaf number in 1995, as well as winter survival: the Pluscarden seedlings tended to be larger and better at surviving

winter conditions. However seed provenance had no effect on percentage root colonisation. Apart from seedling condition and leaf number in 1995, there were no significant interactions between seed provenance and endophyte species.

9.4.3. Effects of inoculation on growth, survival and mycorrhizal colonisation (H_03).

Inoculation with GI lead to a significant amount of root colonisation (30%) compared to GM (5%), and this was associated with significant improvements in height (+ 115%), leaf number (+ 106%) and shoot dry weight (+ 140%) compared with the non-inoculated seedlings. Seedlings inoculated with GM were smaller (-13%); had fewer leaves (-13%) and lower shoot dry weight (-33%) than the non-inoculated seedlings. In the second year (1996) the results were even more noticeable: only GI had sufficient infective propagules to re-establish infections (23% root colonisation). In 1996 GI inoculated seedlings were nearly twice as tall (150 mm) as either GM or control seedlings (80 mm), and had nearly twice the fresh root mass (X 1.7). This increase in root mass would have implications for plant establishment.

Nearly four times as many GI seedlings survived the winter (40%) compared to either GM (13%) or the control plants (10%). The GI treatment was associated with a significant amount of root colonisation (30%); the control and GM seedlings less than 1% root colonisation. The higher level of colonisation was associated with nearly double the height and root mass, and 40% more leaves, compared with either the non-inoculated or GM seedlings.

In GI, increasing the dosage of inoculum given to each seedling by a factor of four (from 1.5 to 5.5 g per pot) lead to nearly ten times the level of root colonisation (6% to 53%), but only 20% more shoot growth compared to the lower dosage of GI. The same

increase in inoculum dosage in GM had no effect on root colonisation but decreased shoot growth by 17% compared to the lower dosage of GM. It is also worth noting that although levels of root colonisation in the lower dosage of GI was comparable to that achieved in either of the GM treatments (all less than 5%), only GM inoculated seedlings overall grew less than the controls. As well as illustrating some of the pitfalls in trying to model root infection data against growth parameters, these results indicate that a higher initial inoculum boost with a suitable endophyte is effective in promoting early growth.

Inoculation initially decreased the growth of the seedlings. During the first four weeks of the experiment, control plants increased their height by 36% compared to less than 15% in any of the endophyte treatments. However during the final two weeks of the first season, the control plants increased their height by only 4%, compared to nearly 11% in the higher (but not lower) dosages of either endophyte species.

9.4.4. *Effects of seed provenance (H_o5).*

Seed provenance had a highly significant main treatment effect on height and leaf number in 1995, as well as winter survival. In 1995 seedlings grown from the Pluscarden seed were 45% taller than those from Loch Naver, and had 15% more leaves. The larger size of the Pluscarden seedlings may account for their greater survival following the cold winter of 1995-6: 40% of the Pluscarden seedlings alive at the end of 1995 were still alive in 1996, compared to only 10% of the Loch Naver seedlings. In 1996 there were no seed provenance effects, perhaps because so few Loch Naver seedlings were still alive.

These results are in contrast to those observed in Experiment One, where seed

provenance had no effects on any of the parameters of growth or mycorrhizal colonisation. The differences observed in the current experiments may be attributable to the uniform nutrient conditions of the sand cultures, which may have highlighted growth differences, in contrast to the more variable soil mixes which may have masked differences.

As in Experiment One, there were no significant effects of seed provenance on percentage mycorrhizal colonisation, or interaction between endophyte species and provenance. Mycorrhizal colonisation was slightly higher in the Pluscarden seedlings (16% across all treatments) than Loch Naver (11%), although this was not statistically significant. Previous studies have indicated interactions between host and endophyte genotypes, for example Morin *et al* [74] noted that apple rootstocks varied in response to inoculation. However, these findings were not corroborated by the present study, perhaps owing to the narrow range of endophyte species and rowan provenances that were studied.

Rowan has a wide geographic, altitudinal and ecotypic distribution (Section 4.5) and so it is not unexpected that some variation should exist between populations. Gillham [140] recorded differences in biomass production between seeds collected from trees growing on limestone and acidic soils. Similarly, Barclay and Crawford [148] found differences in relative growth rate between seedlings grown from seed collected from high and low altitude sites, and Popov [191] observed variation in a range of morphological and fruit characters along a latitudinal gradient in Russia. More recently, Hillebrand and Rosenberg [192] noted differences in shoot diameter increments with altitude, and significantly, isozyme differences between ecotypes, suggesting the involvement of genetic variation. These findings suggest that rowan has much potential for genetic

improvement. However, it should again be emphasized that the seed provenances used in this experiment were selected on the basis of seed availability and germination rates, rather than any systematic selection from particular habitats. Attempts were made to collect seed from different locations throughout Scotland (including the Ballachulish sites used by Barclay and Crawford), but either seed was inaccessible, already predated by birds, or else failed to germination in sufficient quantities for the experiment.

9.4.5. *Exploration of endophyte effects (H_04).*

The most significant findings of the present study were the differences in effectiveness between GI and GM; the effects of initial inoculum dosage, and the effect of inoculation on winter survival.

Endophyte Species

Several explanations are possible concerning the differences in performance between the endophyte species:

1. the GM inoculum was of a poorer quality, or contained fewer infective propagules than that of GI.
2. a preferential host-endophyte association exists between rowan and GI
3. AMF substrate preferences: the GI inoculum was better adapted to sand cultures
4. GM requires a longer period in which establish infections

No initial assessments were made of inoculum infectivity, and so initial quality and propagule level may only be speculated. The inoculum was donated by a commercial company, which implemented its own quality-control procedure, and stated that their products had a 2 year shelf life, when stored in cool, dry conditions. No more may be speculated on this aspect of quality control, although it does have important

implications for anyone considering the use of commercial inoculants. What guarantee does the end-user have that products contains a sufficient quantity of viable propagules of the specified AMF strains; that they are free of contaminating organisms, and not just 'snake-oil'?

Preferential host-endophyte associations have been reported by other researchers, although their results may also have been compounded by endophyte adaptations to particular substrates, and the time course of root colonisation. Kormanik *et al.* [77] grew a range of hardwood species in fumigated soil inoculated with a range of AM fungi. Overall, *G. fasciculatus* promoted growth to a greater extent than any other AMF, although that may have been attributable to the higher initial quantity of inoculum applied: 6600 spores of GF per micro-plot, compared to 2650 spores applied in a mixture of *Gigaspora etunicatum* and *G. mosseae*. They also suggested that the *G. etunicatum* isolate used in their experiments, was adversely affected by high soil temperatures and phosphate levels.

Morin *et al.* [74] noted in apple seedlings grown in steam-sterilised soil, that *G. versiforme* increased height and biomass to a greater extent than either *G. intradices* or *G. aggregatum*, despite being slower at root colonisation and causing an initial growth depression. Hooker *et al.* [42] studied the effects of AMF on root system morphology in poplar: *Glomus* E3 increased secondary root length by almost 200% compared to controls, using *Scutellospora calospora* root length only increased by only 30%. Differences between the AM fungi were related to their ability to colonise roots: overall root colonisation in *Glomus* E3 was considerably higher (22%) than in *S. calospora* (7.8%). Berta *et al.* [43] found that although *G. mosseae* was quicker to colonise cherry roots (*Prunus cerasifera)* than *G. intradices*: 30% colonisation in 20 days, compared to

10%; percentage colonisation was ultimately higher in GI (90%) than GM (70%). Stem weight was also higher in GM (650 mg) than GI (450 mg), and there were differences in foliar P concentration: GM maximised P at day 35, GI at day 50, although neither of these parameters were statistically significant.

AMF-substrate preferences have also been recorded by various researchers. The literature review (Section 3.4) suggested that AMF are not homologous organisms, but demonstrate inter- and intra-specific variation with respect to environmental and edaphic conditions, particularly pH, nutrients and moisture status. No information was available on the isolates used in the present study, although it is conceivable that GM was not adapted to the sand cultures used in the present study. However, the study by Berta *et al* [43] also used GI and GM in sand cultures, and although there were small differences between the fungi, neither appeared to be inhibited by the substrate.

The final explanation for endophyte species differences concerns the time-course of AMF effects. Although percentage infection was only assessed at the end of the season, analysis of seedling growth rate throughout the season indicated differences between the endophyte treatments and controls. At the start of the experiment, the GM seedlings were slightly smaller (14 mm) than those which were inoculated with GI (18 mm), suggesting some bias in the way that the treatments were allocated. However the rate of growth between the GI and GM treatments did not differ throughout the first year of the experiment. In the first 4 weeks the rate of growth was significantly less in both GM and GI compared to the non-inoculated controls, suggesting that inoculation lead to an initial growth depression, attributable to a carbon drain incurred as the AMF became established within the roots. This phenomenon has been widely observed by other studies. Fitter [238] observed that AMF may consume up to 10% of host carbon products.

Morin *et al.* [74] noted an initial growth depression in apple rootstocks inoculated with *G. versiforme*, which was slower to colonise host roots than either than *G. intradices* or *G. aggregatum.* A decline of 5-7% in growth, was recorded by Graham and Eissenstat [72] using citrus rootstocks grown under optimum P conditions. However in the current study, by the final two weeks of monitoring in 1995, inoculated seedlings were growing at a significantly greater rate than control plants, particularly those which had received the higher dosage of inoculum (Figure 9.3). Perhaps, if the experiment had been set up earlier in the season, allowing the seedlings to grow for longer than the observed 2 months, it is possible that the GM seedlings might have caught up with GI in terms of both growth and percentage infection.

Effects on survival.

Inoculation with GI had a dramatic and unexpected effect on survival between the growing seasons, which may be attributed to differences in stress or cold-tolerance between the endophyte treatments. In June 1996, only 25 out of the178 (14%) seedlings alive at the end of September1995 were still alive, of which, 16 (40%) had been inoculated with GI. As mentioned before, winter 1995-6 was exceptionally cold with temperatures remaining below –20°C for over a week in Edinburgh. It is therefore possible that frost-kill may have been responsible for some of the seedling mortality. However as vitality was not assessed between leaf-fall in September and bud break in Spring 1996, the exact cause and timing of mortality can only be estimated. Mean seedling height in 1995 in the best treatments was less than 3 cm; most commercial nurseries would expect growth of around 30 cm during the first season. This would tend to indicate that the seedlings were grown under less than optimal conditions either as a result of drought or nutrient stress – seedlings were grown in a free-draining coarse sand, receiving only 25 to 50 ml of a half-strength liquid feed per week No additional

irrigation was applied. Seedlings grown under stress, although initially appearing to survive, may be physiologically unable to survive the demands of bud-break, and so mortality may not become apparent until the following spring. This phenomena of increased plant death around bud break was observed by Gilbertson *et al* [10] and may be attributed to drought during the previous autumn or spring deoxygenation. In the present study it was also observed that surviving seedling tended to be significantly larger than those which did not. During the same period, fewer seedlings died in the soil experiment (Chapter 8), which experienced the same cold conditions, although the seedlings were taller, as well as a year older.

The effects of cold on AMF have been little addressed by previous researchers. Visser *et al.* [179] found that *Elaeagnus commutata* and *Shepherdia canadensis* dually inoculated with AMF and *Frankia* experienced greater winter kill than non-inoculated plants, which they attributed to insufficient winter hardening, and physiological and nutritional differences between treatments. However Paradis *et al* [243] suggested that AMF improved cold-tolerance, as they observed less dark red pigment (associated with cold-induced oxidative stress) in mycorrhizal wheat cultivars compared to non-mycorrhizal following short-term exposure to –5°C.

Rowan is an extremely hardy tree: it is often used as a rootstock for a range of rosaceous woody plants in Eastern Europe because of it "..complete resistance to the severest of winter conditions" [149]. McEvoy and McKay [141] estimated root hardiness down to –6°C in bare-root 3 year old seedlings, using 50% root electrolyte leakage (REL_{50}) as an indicator of damage caused by freezing. These studies would appear to suggest that is unlikely that rowan would be susceptible to winter kill. The roots are also tolerant of desiccation, as reported by Dutton and Bradshaw [17]. The findings of this

study, with regard to increased winter survival due to inoculation are therefore of interest to practitioners and merit further study.

9.4.6. Improvements to experimental design and suggestions for future experiments.

A major shortcoming of this experiment was that inoculum quality and propagule levels were not assessed at the start, in order that the causes of the poor effects of the GM inoculum might have been explored. More information about the time course of root colonisation and inoculum effects might have been gained from more frequent assessments of mycorrhizal colonisation during the growth season.

Were the chosen growth parameters the most appropriate and informative? Height and shoot dry weight are indicators of shoot biomass production; however the inclusion of stem diameter would have given an indication of 'sturdiness quotient', one of the quality indicators used by the Forestry Commission to predict establishment success. Some measure of plant physiology (carbohydrate levels or root electrolyte leakage) might have allowed the cold-effects to be explored. A greater analysis of root system architecture using image analysis, or some less sophisticated method of assessing root length and branching, would also have permitted more understanding of the mechanistic effects of AMF on rowan growth and survival.

9.4.7. Conclusions.

This experiment has highlighted the potential benefits, in terms of enhanced early growth and winter survival, to be gained form inoculating woody plants such as rowan with suitable AMF. GI appeared to be the most effective endophyte: a higher initial dosage of GI inoculum lead to a significant amount of mycorrhizal colonisation and was associated with significant increases in shoot height, biomass and root mass. However

it is not certain whether the GM inoculum contained as many viable propagules, as this was not assessed, or whether some incompatibility existed between rowan and GM. There is also the possibility that GM required a longer establishment period before beneficial effects become apparent. The effects of inoculation on winter survival were unexpected and merit further study.

With regard to the null hypotheses proposed at the start of the study:

H_o3. AMF have no effect on the early growth of rowan: reject.

H_o 4. All AMF species are equally effective endophytes of rowan: reject.

H_o 5. There are no interactions between AMF and host genotype (seed provenance): accept.

It had been intended to use some of the inoculated seedlings from this experiment for a field study, to explore whether the effects of inoculation were sustained after planting. After all, the 'acid test' of plant quality is whether the plant survives to fulfil a function in the landscape. It is possible that an endophyte adapted to growing in sand cultures may not perform as well either in soil or in nursery conditions under a higher fertiliser regime. Had more of the experimental plants survived, this would have had particular relevance to those responsible for growing and specifying trees in the landscape.

CHAPTER TEN.

EXPERIMENT THREE: THE EFFECTS OF PRE-PLANTING INOCULATION WITH AMF ON THE GROWTH AND SURVIVAL OF NATIVE TREES ON A RECLAIMED LANDSCAPE SITE.

10.1. INTRODUCTION.

The second experiment indicated that the growth and survival of rowan seedlings maintained in sand cultures under ambient glasshouse conditions, was improved following inoculation with the arbuscular mycorrhizal fungi *Glomus intradices.* After two seasons, inoculated seedlings were taller (200%), had a greater root mass (84%) and increased survival (400%) compared to non-inoculated controls. However, it was unclear whether these early growth benefits would translate into improved survival and post-planting performance under landscape site conditions. It was also unknown whether the inoculum would persist in a field soil, which contained indigenous populations of AMF.

It had been intended to use seedlings from the inoculation experiment. However, only 25 of the original 250 seedlings survived the first winter and so it was necessary to use commercially grown material from a local tree nursery. Cell-grown material was used as it could be inoculated prior to planting with minimal disturbance to the root system. A range of native species (see Appendix 1) known to associate with AMF were chosen in order to expand the relevance of the experiment to the landscape industry: rowan (*Sorbus aucuparia* L.), ash (*Fraxinus excelsior* L.) and wild cherry (*Prunus avium* L.). As in the previous experiment two species of AMF were used: *Glomus intradices* and *G. mosseae,* in order to investigate whether there were any host-endophyte interactions.

Kendle [14] observed that little was known about the survival of AMF on urban soils, and suggested that plants might be too stressed to form mycorrhizal associations. Urban soils differ considerably from those found in natural ecosystems (Section 2.2) and tend to lack sufficient populations of AMF and other beneficial soil micro-organisms (Section 3.4 and 3.5). Danielson and Visser [122] published one of the few studies that considered the mycorrhizal status of landscape woody plants used in reclamation schemes, and noted that few one-year old plants were mycorrhizal at the time of planting. This was due to a combination of factors including high fertility levels, lack of inoculum in the substrate and inefficient dispersal of propagules, although they observed that plants that spent some time growing in outdoor conditions, might become erratically mycorrhizal. Plants would therefore be reliant on AMF propagules present in the planting site, and if dependent on AMF would benefit from artificial inoculation, particularly if the site was highly disturbed and lacking in symbionts.

In the survey of nurseries and sites conducted in the present study (Section 7), AMF colonisation levels of around 30% were recorded in rowan in nurseries, and 40% on sites. However the nursery plants were grown outdoors, and although the sites varied in disturbance, plants had been established between 2-7 years.

Few AMF trials have been conducted under realistic landscape conditions. Plenchette *et al* [73] demonstrated growth benefits in non-sterile soil, although growing conditions were not particularly stressful. Johnson and Crews [244] observed increased root and shoot growth in inoculated *Rhododendron simsi* transplanted to sandy loan soils and only irrigated at the point of wilting. Visser *et al* [79] also recorded increased root and shoot growth in actinorrhizal shrubs dually inoculated with AMF and *Frankia*, although winter kill owing to insufficient hardening was also higher. Morrison *et al* [80] recorded

few benefits following transplantation of inoculated woody plants into fertile field sites. Similarly, Delisle [81] reported no differences in survival, height or root collar diameter between inoculated and non-inoculated *Fraxinus pennsylvanica* seedlings transplanted onto a range of abandoned fields and clear-cut forestry areas. These studies offer few guidelines to landscape practitioners considering inoculation.

St John [134] considered that a commitment to modify nursery and landscape practices such fertiliser regimes, irrigation and substrate was necessary, if the use of AMF was to become a workable technology. Koch *et al.* [82] observed that mycorrhizal apple seedlings maximised growth at lower levels of applied phosphate than non-inoculated plants. There is also evidence that supra-optimal levels of fertiliser application are deleterious to AMF by reducing host-dependency on the symbiosis. It was therefore decided to investigate the effects of post-planting fertiliser application on endophyte functioning.

The field trial was therefore set up in April 1998 on a reclaimed oil-shale bing in West Lothian, Scotland, managed by the Central Scotland Woodland Trust, to test the following null hypotheses:

- $H_0 4$: All AMF species are equally effective endophytes of rowan
- $H_0 5$: There are no interactions between AMF and host genotype (tree species)
- $H_0 6$: Environmental conditions have no effect on the functioning of AMF.

10.2. MATERIALS AND METHOD.

10.2.1. Preparation of plant material

In February 1998, one-year old cell-grown trees were graded by nursery staff and inoculated with AMF at the nursery of Alba Trees, East Lothian. All trees were of an acceptable commercial standard and had a minimum height of 30cm and stem diameter of 4 mm. Due to the late start of the experiment and shortage of material at the nursery, it was not possible to use Scottish provenances of all the species. Seed origin was as follows: Ash: Shropshire; Cherry: Pencaitland (East Lothian), Rowan: Roxburgh (Borders).

The treatments consisted of non-inoculated controls, and inoculation with either *G. intradices* (GI) or *G. mosseae* (GM). Each tree to be inoculated was given 5g (the high dosage used in Experiment Two) of the appropriate inoculum, on a clay-based carrier, 8/16 formulation supplied by MicroBio Ltd, which was sprinkled onto the root system as the trees were re-containerised into 175 cm^3 'Rootrainers'. A peat-based compost supplied by the nursery was then used to fill up any gaps in the cells and ensure close contact between roots and inoculum. The containers were then closed and packed into trays of 32. Non-inoculated trees were repacked using the compost only.

The trees were then placed on pallets outside in the nursery until required for planting out on site in April 1998. Post-inoculation assessments were made in the nursery of height (in mm) and stem diameter (in mm) at 10 cm above root collar using dial calipers. At the time of planting, 8 trees from each species/AMF treatment combination (a total of 72 trees) were retained and assessed for percentage mycorrhizal colonisation (Section 6.3 and 6.4) using a bulk-staining method.

10.2.2. Site selection.

The landscape site was selected following contact with local landscape companies and organisations involved in land reclamation. The following site selection criteria were used:

- within 50 mile radius of Edinburgh
- away from housing and schools to reduce the risk of vandalism
- easily accessible
- that it would remain clear of other plantings for several seasons
- establishment was expected to be 'difficult'. This included nutrient or water deficiency, and poor soil structure, but excluded sites where toxicity or contamination problems were expected, as these were considered beyond the scope of the study.

The only available site to meet these criteria was Stankards Bing, near Livingston, West Lothian (NT 066 710), a former oil-shale bing which had been rehabilitated the previous year and planted with a range of native tree species (rowan, ash, hawthorn, birch, oak and alder). The site was managed by the Central Scotland Woodland Trust. The site had been graded, covered with subsoil and contoured. The slopes were fairly steep, and some surface water was present. The plot selected for the trial had no vegetation apart from a few scattered weed species. No ground preparation was undertaken prior to planting.

10.2.4. Experimental design.

A split plot design was chosen to take account of variation in slope, aspect and soil conditions. The site was divided into 9 plots; each AMF treatment and tree species combination (three tree species, three AMF treatments) was repeated within each plot.

To minimise contamination between AMF treatments, the plots were further divided into three sub-plots of three rows: each subplot contained a single endophyte treatment, with one row for each tree species. Tree spacing was 1 m within and between rows; 1.5 m between treatment subplots, and 2 m between plots. The site plan is shown in Figure 10.1. Planting took place in early April 1998 in extremely wet conditions.

In addition, a fertiliser treatment was applied post-planting to the plots (3 plots per treatment). This consisted of no fertiliser, half the recommended dose (15 g per tree) and the full recommended dose (30 g per tree). The fertiliser was Sierra Blend Yellow (15 : 9 : 9 : 3), a granular, slow-release formulation for trees and shrubs, which is low in phosphate), obtained from Scotts, UK Ltd. The fertiliser was applied by making a hole adjacent to the trees using a bulb planter, and placing the fertiliser within it. This was considered to be the most appropriate method for the fertiliser granules, owing to the steep slope, and capped nature of the soil surface.

Three of the plots were sampled for nutrient status (total nitrogen, potassium, phosphate, magnesium) and pH. Samples were sent to a commercial soil testing service (SAC, Edinburgh). Financial constraints limited the number of samples it was possible to assess. Results are indicated below in Table 10.1.

Table 10.1. Soil analysis results for Stankards Bing, West Lothian. (VL = very low; L = low; H = high).

Plot	Total Nitrogen (mg kg^{-1})	Phosphate (mg l^{-1})	Potassium (mg l^{-1})	Magnesium (mg l^{-1})	PH
1	630	1.4 (VL)	65.0 (L)	326 (H)	8.0
5	580	1.6 (VL)	69.6 (L)	376 (H)	8.1
9	600	1.4 (VL)	68.4 (L)	316 (H)	8.1

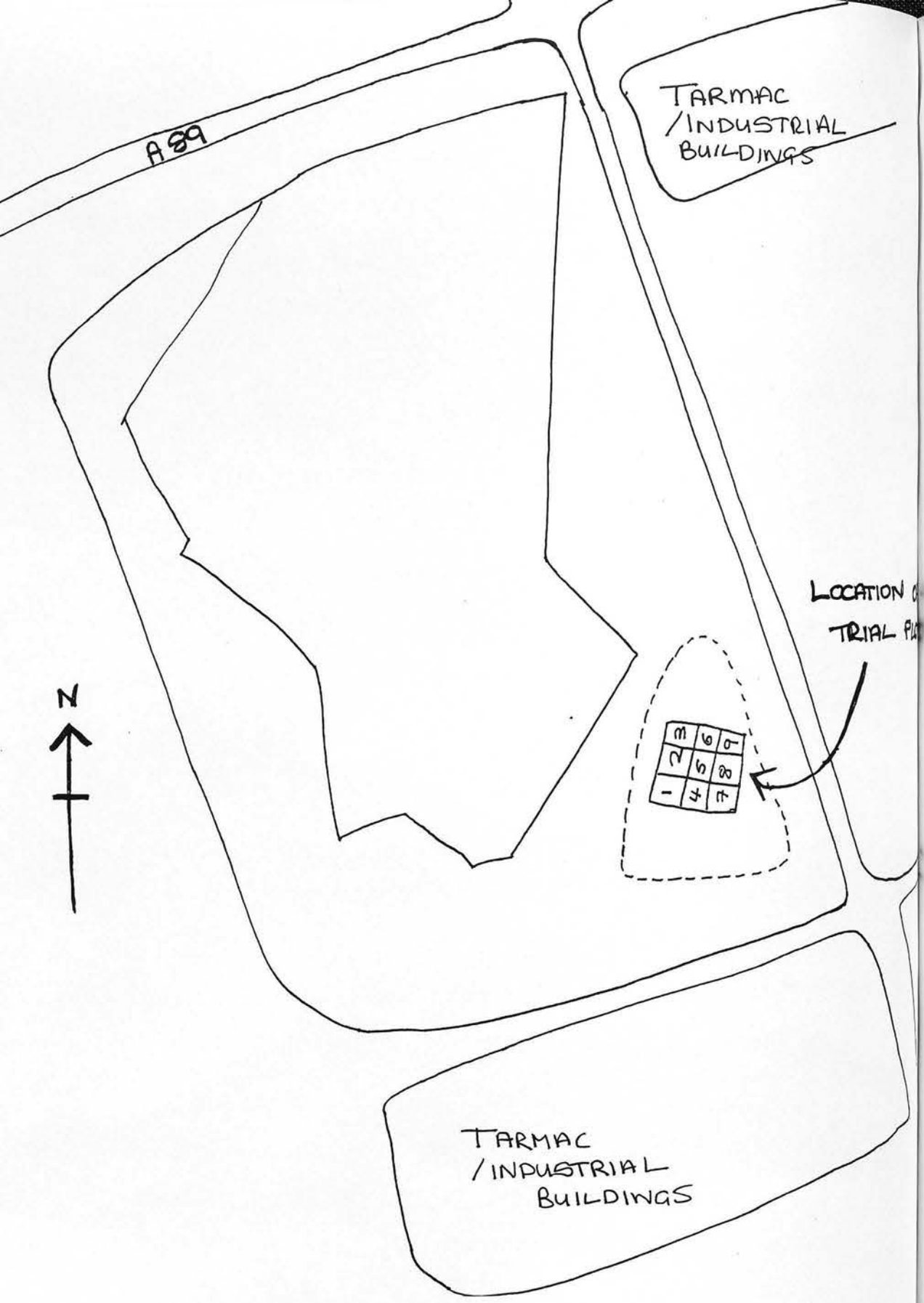


FIGURE 10·1a. SITE PLAN OF STANKARDS BING CNT 064713
(SCALE 1:2500)

Figure 10.1b. Experimental plan for Stankards Bing.

12 m

10 m

Plot 1			Plot 2			Plot 3		
C	GI	GM	GI	GM	C	GM	C	GI
Rowan	Rowan	Rowan	Rowan	Rowan	Rowan	Rowan	Rowan	Rowan
Ash	Ash	Ash	Ash	Ash	Ash	Ash	Ash	Ash
Cherry	Cherry	Cherry	Cherry	Cherry	Cherry	Cherry	Cherry	Cherry

Plot 4			Plot 5			Plot 6		
GM	GI	C	GM	C	GI	GI	GM	C
Ash	Ash	Ash	Ash	Ash	Ash	Ash	Ash	Ash
Cherry	Cherry	Cherry	Cherry	Cherry	Cherry	Cherry	Cherry	Cherry
Rowan	Rowan	Rowan	Rowan	Rowan	Rowan	Rowan	Rowan	Rowan

Plot 7			Plot 8			Plot 9		
GI	GM	C	GM	GI	C	GM	C	GI
Cherry	Cherry	Cherry	Cherry	Cherry	Cherry	Cherry	Cherry	Cherry
Rowan	Rowan	Rowan	Rowan	Rowan	Rowan	Rowan	Rowan	Rowan
Ash	Ash	Ash	Ash	Ash	Ash	Ash	Ash	Ash

Key to Experimental Treatments:
Plot Level: Fertiliser Treatment

No fertiliser	Half recommended dosage (15 g plant^{-1})	Full recommended dosage (30 g plant^{-1})

Subplot Level: AMF Treatment

C	GI	GM
Control	G. intradices	G. mosseae

Row Level: Tree species
Rowan, Ash, Cherry: 8 plants per row

At the end of the first season, soil bulk density was also assessed using a volumetric method. A core of soil was removed using a steel cylinder of a known volume (209 cm^3). Two samples were taken from each of the nine plots. The soil cores were dried in an oven at 80°C for 5 days, until no further change in dry weight was observed. Bulk density was then calculated by dividing the dry weight by the original volume of each core. Results of bulk density are presented below in Table 10.2.

Table 10.2. Soil bulk density (g cm^{-3}) in September 1998.

Plot	Soil Fresh Weight (g)	Soil Dry Weight (g)	Percentage Moisture	Bulk Density (g cm^{-3})
1.00	414.64	356.02	14.74	1.70
1.00	408.35	350.23	14.80	1.68
2.00	430.64	373.33	13.67	1.79
2.00	387.84	337.58	13.03	1.62
3.00	420.87	357.30	16.07	1.71
3.00	400.09	344.64	14.26	1.65
4.00	425.57	368.98	13.64	1.77
4.00	452.85	392.22	13.85	1.88
5.00	399.80	344.30	14.32	1.65
5.00	413.54	360.49	12.93	1.72
5.00	406.88	349.45	14.68	1.67
6.00	401.51	344.84	14.57	1.65
6.00	434.49	373.97	14.48	1.79
7.00	422.65	355.56	17.10	1.70
7.00	427.20	371.97	13.18	1.78
8.00	398.13	343.13	14.21	1.64
8.00	443.30	380.68	14.81	1.82
9.00	413.69	347.84	17.15	1.66
9.00	385.53	329.00	15.27	1.57
				Mean Bulk Density 1.71 (g cm^{-3}) SD = 0.08

Soon after planting rabbit damage became apparent on some of the trees and, at the recommendation of the site manager, spiral tree guards with bamboo stakes were applied to the trees by a contractor. However this protective treatment caused additional damage to the stem leaders, and the plastic guards were too large for many of the trees, causing them to fall over. It was also difficult to measure the trees without moving the guards, contributing to even more stem damage.

Dependent variables

At the end of the first growing season, in September 1998, assessments were made of tree survival, height, stem diameter and condition. The latter was a subjective assessment to take account of damage caused by rabbit grazing, the tree guards, as well as any dieback caused by drought:

0 = dead

1 = severe dieback of main shoot

2 = some dieback of main shoot

3 = limited growth/some damage to main shoot

4 = good growth with no damage to main shoot.

At the same time, 1 tree of each treatment/species combination from each plot (total of 81 trees) was harvested for determination of shoot and root fresh and dry root weight. All the trees sampled in this way were removed from the same position (number 3) in each row, or the nearest living tree. A small amount of root was removed from each root system, weighed, and then used for determination of mycorrhizal colonisation using a bulk-staining method (Section 6.3 and 6.4). The final dry weight of the roots system was adjusted to take account of the root tissue removed. Tree survival was also assessed in May 1999, just after bud burst.

10.3. RESULTS

10.3.1. Statistical analysis

The field experiment was set up as a split-plot design, and an analysis of variance appropriate to split-plot designs was used to examine the results. The experimental area (main plot) was divided into 9 sub-plots. The fertiliser treatment comprised 3 levels applied between subplots; there were 3 sub-plot replicates of each fertiliser treatment. The tree species and mycorrhizal treatments also comprised 3 levels and were applied within each sub-plot. In this way, the fertiliser treatment had 2 degrees of freedom, and a different error term from the tree species and mycorrhizal treatment. The method of statistical calculation is described in Section 6.6. Treatment factor levels and dependent variables are given in Table 10.3.

Table 10.3. Treatment factor levels and dependent variables.

Treatment Factor Levels

- **Main Plots**
  - Fertiliser: 3 levels (no fertiliser, half-dosage; full dosage).
- **Subplots**
  - Tree species : 3 levels (ash, cherry, rowan)
  - Mycorrhiza : 3 levels (non-inoculated controls, *G. intradices, G. mosseae)*
  - Interaction terms

Dependent Variables

- **Pre-planting**
  - Height (in cm)
  - Stem diameter (in mm)
  - Mycorrhizal colonisation (%)
- **After one growing season**
  - Height (in cm)
  - Stem diameter (mm)
  - Root fresh weight (g)
  - Root dry weight corrected (g)
  - Shoot dry weight (g)
  - Mycorrhizal colonisation (%)

In the following section, pre-planting and end of year dependent variables will be presented separately. The analysis of variance will be given, followed by the significant interaction and main treatment effects.

10.3.2. Pre-planting assessments

Analyses of variance for height, stem diameter and mycorrhizal colonisation, are given below in Table 10.4. Height and stem measurements were taken prior to planting. Percentage colonisation was assessed destructively and so applies to a set of plants not used in the main experiment.

Table 10.4. Analysis of variance for pre-planting assessments (F-statistic, degrees of freedom, significance[1].

	Height	**Stem Diameter**	**Percentage Colonisation [2]**
Main Plot			
Fertiliser	0.42, 2 ns	0.315, 2 ns	N/A[1]
Sub Plots			
Species	**30.38, 2 *****	**26.03, 2 *****	**22.086, 2 *****
Mycorrhiza	2.19, 2 ns	2.68, 2 ns	0.152, 2 ns
Fertiliser* Species	0.43, 4 ns	1.427, 4 ns	N/A
Fertiliser *Mycorrhiza	2.06, 4 ns	0.202, 4 ns	N/A
Species *Mycorrhiza	**5.04, 4 ****	0.670, 4 ns	N/A
Species *Mycorrhiza *Fertiliser	1.42, 8 ns	1.473, 8 ns	N/A

[1] *** = very highly significant (P<0.001); ** highly significant (P<0.01)
* = significant (P<0.05); ns = not significant
[2] Percentage colonisation was assessed on a sample of trees not used in the planting and therefore outwith the split-plot analysis.

This analysis indicated that there were very highly significant differences between the tree species in height and stem diameter (P<0.001). The cherry trees tended to be slightly taller (35 cm) than the ash (33 cm) or rowan trees (30 cm). The cherry trees

also had a slightly greater stem diameter (4.9 mm) than either ash (4.5 mm) or rowan (4.6 mm). There was also a significant species * mycorrhiza interaction ($P<0.01$) for height, suggesting that there may have been some bias in the allocation of trees between mycorrhizal treatments. For this reason, analysis of covariance was used to examine height differences in subsequent analyses. Results are given by species and mycorrhizal treatment in Table 10.5.

Table 10.5. Pre-planting assessments of height, stem diameter and percentage root colonisation. Figures in brackets indicate standard error of mean and number of plants sampled.

1. Height (cm)				
	Ash	*Cherry*	*Rowan*	Mean
Control	32.1 (.66, 72)	36.8 (.73, 72)	30.5 (.58, 72)	33.1 (.41,216)
GI	32.3 (.80, 72)	34.8 (.68, 72)	29.2 (.42, 72)	32.1 (.41, 216)
GM	33.4 (.59, 72)	32.2 (.59, 72)	30.5 (.58, 72)	32.0 (.35, 216)
Mean	32.6 (.40, 216)	34.7 (.40, 216)	30.0 (.29, 216)	
2. Stem diameter (mm)				
	Ash	*Cherry*	Rowan	Mean
Control	4.4 (0.08, 72)	4.9 (0.10, 72)	4.6 (0.06,72)	4.6 (.05, 216)
GI	4.5 (0.09, 72)	4.9 (0.07, 72)	4.5 (0.06,72)	4.6 (.05, 216)
GM	4.6 (0.09, 72)	5.0 (0.08, 72)	4.7 (0.08,72)	4.8 (.05, 216)
Mean	4.5 (0.05, 216)	4.9 (0.05, 216)	4.6 (0.04, 216)	
3. Percentage root colonisation				
	Ash	*Cherry*	*Rowan*	Mean
Control	0.3 (0.32, 8)	11.4 (7.59, 8)	38.7 (4.14,8)	16.8 (21.4, 24)
GI	0.0 (0.00, 8)	2.7 (1.31, 8)	22.8 (7.08,8)	8.5 (15.3, 24)
GM	10.6 (8.83, 8)	4.6 (3.05, 8)	28.6 (6.68,8)	14.6 (20.7, 24)
Mean	3.7 (3.00,24)	6.2 (2.75, 8)	30.0 (3.64,24)	

There was a significant difference ($P<0.001$) between tree species in mycorrhizal colonisation: rowan had significantly greater colonisation (30%) than either cherry (6%) or ash (4%). However, at this stage there were no significant differences in colonisation between the mycorrhizal treatments: controls (17%), GI (8%) and GM (15%). Mycorrhizal colonisation was assessed 8 weeks after inoculation, and these results

suggest the possibility of pre-existing colonisation by indigenous AMF, or that inoculation had not been successful at that stage.

10.3.3. End of year (1998) assessments.

Analysis of variance for height (using pre-planting height as a co-variant), stem diameter and condition at the end of the 1998 growing season are given below in Table 10.6. These indicate few significant main treatment or interaction effects by the end of 1998. Fertiliser had no main treatment or interaction effects and so results will be given for tree species and mycorrhizal treatment only. Tree species had a highly significant effect on height ($P<0.01$), shoot extension ($P<0.01$), stem diameter change ($P<0.001$) and condition ($P<0.001$). Mycorrhizal treatment had a significant effect ($P<0.01$) on stem diameter change only. There was a significant interaction between tree species and mycorrhizal treatment for condition ($P<0.05$). These parameters will be presented individually by tree species and mycorrhizal treatment only.

Table 10.6. Analysis of variance for end of growing season 1998. (F Statistic, degrees of freedom, significance[1]).

	Total Height [2]	**Height Change**	**Total Stem Diameter**	**Stem Diameter Change**	**Condition**	**Root Fresh Weight**	**Root Dry Weight (Corrected)**	**Shoot Dry Weight**	**Percentage Colonisation 2**
Main Plot									
Fertiliser	0.29, 2 ns	0.535, 2 ns	0.057, 2 ns	0.261, 2 ns	0.180, 2 ns	0.386, 2 ns	0.597, 2 ns	0.495, 2, ns	0.675, 2 ns
Sub Plots									
Species	**6.51, 2 ****	**7.280, 2 ****	0.468, 2 ns	**11.769,2 *****	**19.444, 2 *****	**10.413, 2 *****	**4.110, 2, ***	1.786, 2 ns	**15.423, 2 *****
Mycorrhiza	0.09, 2 ns	0.027, 2 ns	1.793, 2 ns	**5.085, 2 ****	2.386, 2 ns	0.223, 2 ns	0.623, 2 ns	0.084, 2 ns	0.774, 2 ns
Fertiliser* Species	1.05, 4 ns	1.085, 4 ns	0.651, 4 ns	0.783, 4 ns	1.328, 4 ns	0.319, 4, ns	0.424, 4, ns	0.301, 4, ns	0.679, 4 ns
Fertiliser *Mycorrhiza	1.15, 4 ns	1.070, 4 ns	1.600, 4 ns	1.104, 4 ns	1.663, 4 ns	1.325, 4 ns	0.327, 4, ns	0.676, 4 ns	0.201, 4 ns
Species *Mycorrhiza	0.62, 4 ns	0.548, 4 ns	0.811, 4 ns	0.034, 4 ns	**2.828, 4 ***	**4.160, 4 ****	1.009, 4 ns	1.194, 4 ns	0.075, 4 ns
Species *Mycorrhiza *Fertiliser	0.61, 8 ns	0.576, 8 ns	0.551, 8 ns	0.923, 8 ns	0.880, 8 ns	0.982, 8 ns	0.383, 8 ns	1.543, 8 ns	0.556, 8 ns

[1] *** = very highly significant (P<0.001); ** highly significant (P<0.01)
*significant (P<.05); ns = not significant

[2] Using pre-planting height as covariant.

Height

Only tree species had a significant effect on height at the end of the growing season, and height change since planting. Ash (39 cm) and cherry (41 cm) were slightly taller than rowan (36 cm) at the end of the year (Table 10.7). When mean shoot extension during the first year was considered, the ash increased in height by nearly 8 cm compared to cherry (4 cm) and rowan 6 cm). Although only the species effects was significant for shoot extension ($P<0.01$), it is worth noting that the cherry inoculated with GI produced the least mean shoot extension (2.3 cm) of any of the species/mycorrhizal treatments. Shoot extension was based on final *living* heights, and was related to the degree of stem damage caused by rabbit grazing, stem guards and frost, as well as any transplanting-related die-back.

Table 10.7. The effect of tree species and mycorrhizal treatment on final height (in cm) and height change during 1998.
Figures in brackets indicate standard error of mean and number of replicates.

1. End of Year Height				
	Ash	*Cherry*	*Rowan*	Mean
Control	40.3 (1.26, 68)	42.8 (1.62, 60)	35.1 (1.33, 54)	39.6 (0.84, 182)
GI	41.0 (1.26, 63)	37.9 (1.93, 45)	35.7 (1.47, 58)	38.3 (0.88, 166)
GM	40.7 (1.51, 62)	36.8 (1.83, 53)	36.3 (1.54, 57)	38.1 (0.94, 172)
Mean	40.7 (0.77,193)	39.4 (1.04,158)	35.7 (0.84, 169)	
2. Shoot Extension				
	Ash	*Cherry*	Rowan	Mean
Control	7.9 (1.12, 68)	5.0 (1.40, 61)	4.6 (1.13, 54)	6.0 (0.72, 183)
GI	8.2 (1.44, 63)	2.3 (1.59, 45)	6.4 (1.48, 58)	6.0 (0.88, 166)
GM	7.0 (1.45, 62)	4.4 (1.56, 53)	5.5 (1.58, 57)	5.7 (0.88, 172)
Mean	7.7 (0.77,193)	4.1 (0.87, 159)	5.5 (0.82, 169)	

Stem diameter

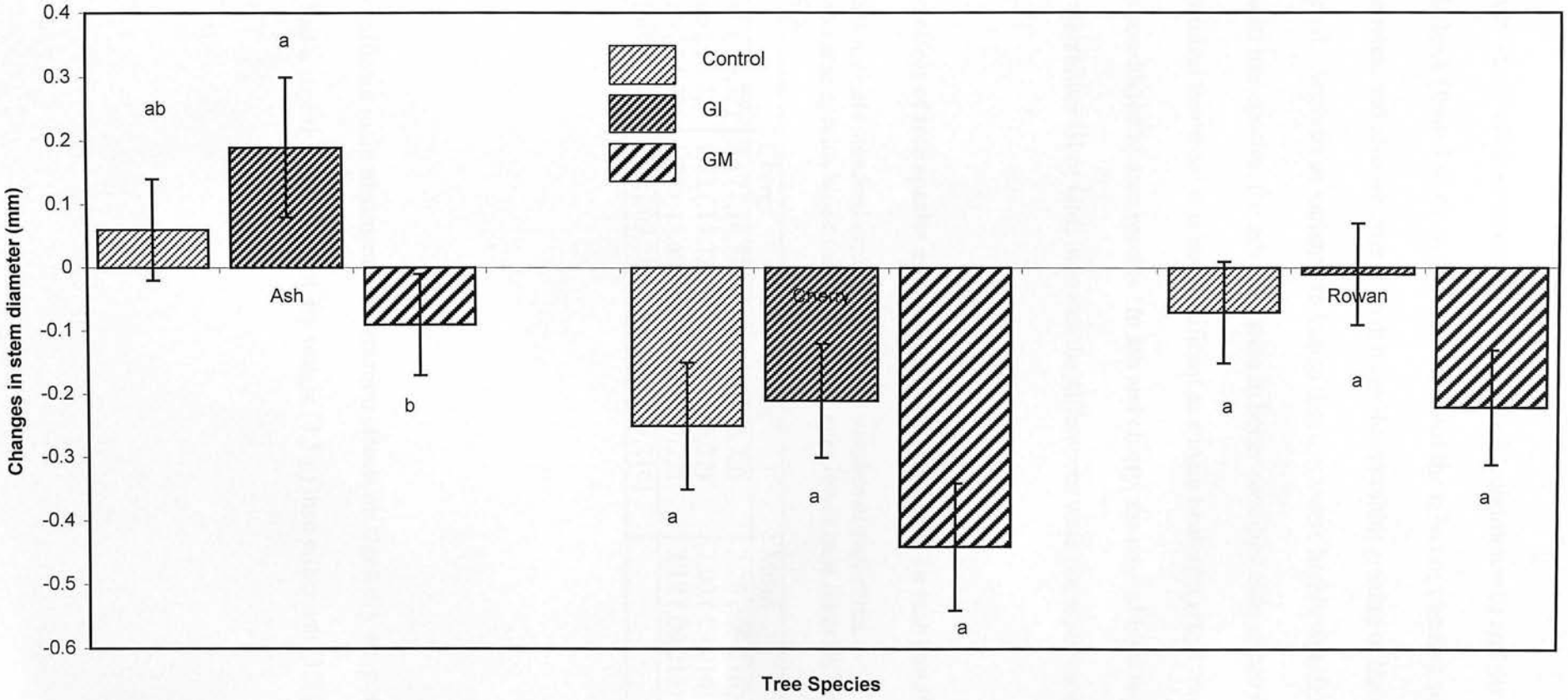
In 1998 there were no significant treatment or interaction effects on stem diameter; tree species and mycorrhizal had significant main treatment effects of treatment on stem diameter change (Table 10.8). The results for stem diameter change, were puzzling in

that stem diameter appeared to decrease for some of the treatments throughout the growing season. Possible causes of this observation will be considered in the discussion. In general it appeared that stem diameter in ash remained more or less the same, but that it decreased slightly in cherry and rowan. Similarly stem diameter was more or less the same in the control trees, and decreased slightly in the GI and GM treatments. Changes in stem diameter are shown in Figure 10.2.

Table 10.8. The effect of tree species and mycorrhizal treatment on end of year stem diameter and stem diameter changes (in mm) in 1998.
Figures in brackets indicate standard error of mean and number of replicates.

1. Stem Diameter				
	Ash	*Cherry*	*Rowan*	Mean
Control	4.5 (0.11, 64)	4.7 (0.08,60)	4.6 (0.10,54)	4.6 (0.06,178)
GI	4.7 (0.11, 63)	4.7 (0.10,45)	4.5 (0.08,57)	4.6 (0.06,165)
GM	4.6 (0.11, 62)	4.5 (0.08,51)	4.5 (0.10,57)	4.5 (0.06,170)
Mean	4.6 (0.06,189)	4.6 (0.05,156)	4.5 (0.05,168)	
2. Change in Stem Diameter				
	Ash	*Cherry*	Rowan	Mean
Control	0.06 (.08,64)	-.25 (.10,60)	-.07 (.08,54)	-.08 (.05,178)
GI	0.19 (.11,63)	-.21 (.09,45)	-.01 (.08,57)	0.01 (.06,165)
GM	-.09 (.08,62)	-.44 (.10,51)	-.22 (.09,57)	-.24 (.05,170)
Mean	0.05 (.05,189)	-.30 (.06,156)	-.10 (.03,213)	

Figure 10.2. The effect of endophyte treatment on changes in stem diameter, March to September 1998.
(Significant differences within species only)



Condition

Tree condition was a subjective assessment of tree survival (condition = 0) and the degree of stem dieback (from 1 to 4). It therefore reflected the effect of planting site conditions on the trees, and also whether stem damage due to rabbit grazing or the tree guards had occurred. Analysis of variance indicated that there were highly significant differences between tree species: the ash trees were in better condition than either rowan or cherry. Mycorrhizal treatment was not significant as a main treatment effect, but was significant when considered by tree species. In ash and cherry, the control trees were in better condition than either GI or GM; in rowan the differences were not significant (Table 10.9).

Table 10.9. The effect of tree species and mycorrhizal treatment on tree condition in 1998.
Figures in brackets indicate standard error of mean and number of replicates.
Treatments within same species block followed by different lower case letter differ at P<0.05 using LSD.

	Ash	*Cherry*	*Rowan*	Mean
Control	2.79 a (.13,72)	2.17 (.14,72) a	1.89 (.16,72)	2.28 (.09,216)
GI	2.58 ab (.15,72)	1.42 (.14,72) b	1.99 (.16,72)	2.00 (.09,216)
GM	2.38 cb (.14,72)	1.75 (.15,69) b	2.21 (.17,72)	2.12 (.09,213)
Mean	2.58 (.08,216)	1.78 (.09,213)	2.03 (.09,216)	

Stem dry weight

There were no significant main treatment or interaction effects on shoot dry weight, although cherry had a slightly greater shoot dry weight (3.5 g) than either ash (3.2 g) or rowan (2.6 g).

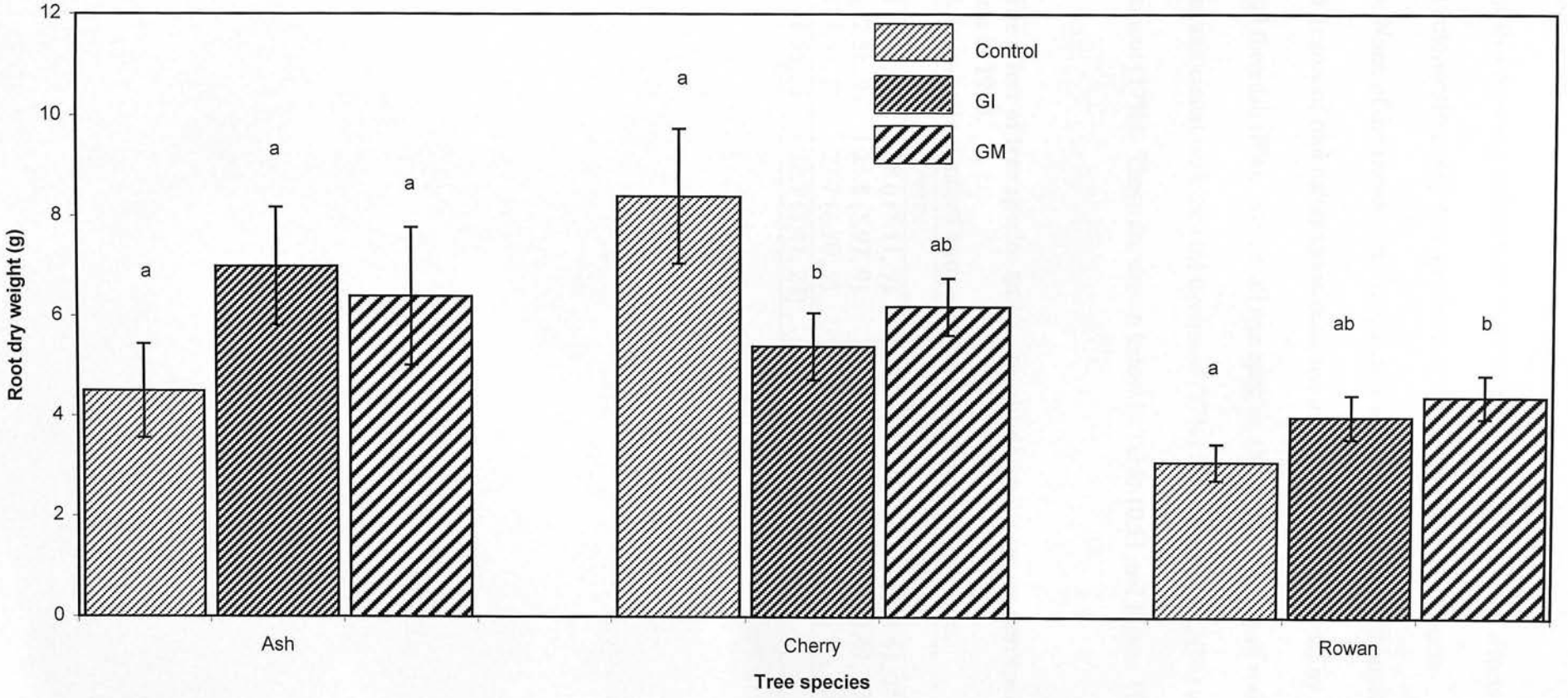
Root Mass

Tree species had a highly significant main treatment effect on root fresh and dry weight: cherry had a greater root mass (6.6 g fresh weight) than either ash (6.3 g) or rowan (3.8 g). There was a significant interaction between tree species and mycorrhizal treatment on root fresh weight only. In ash the greatest fresh root mass was associated with the GI treatment (7.9 g); in cherry with the control treatment (8.4 g) and in rowan with the GM treatment (4.4 g). These results are given below in Table 10.10 and Figure 10.3.

Table 10.10. The effect of tree species and mycorrhizal treatment on shoot and root mass in (in g) 1998.
Figures in brackets indicate standard error of mean and number of replicates.

Factor	Shoot dry weight	Root fresh weight	Root dry weight
Ash			
Control	2.8 (.42, 9)	4.5 (.94, 9)	1.9 (.31, 9)
GI	3.5 (.68, 9)	7.9 (1.18, 9)	2.9 (.57, 9)
GM	3.5 (.69, 9)	6.4 (1.38, 9)	3.4 (.90, 9)
Mean	3.2 (.35, 27)	6.3 (.71, 27)	2.7 (.37, 27)
Cherry			
Control	4.4 (.68, 8)	8.4 (1.34, 9)	3.9 (.73, 6)
GI	2.7 (.33, 9)	5.4 (.67, 9)	2.4 (.20, 9)
GM	3.5 (.54, 9)	6.2 (.57, 9)	2.9 (.33, 9)
Mean	3.5 (.32, 26)	6.6 (.57, 27)	3.0 (.25, 24)
Rowan			
Control	2.5 (.27, 9)	3.1 (.36, 9)	1.3 (.18, 8)
GI	2.9 (.37, 9)	4.0 (.44, 9)	1.5 (.19, 8)
GM	2.5 (.44, 9)	4.4 (.43, 9)	1.7 (.16, 9)
Mean	2.6 (.21, 27)	3.8 (.25, 27)	1.5 (.10, 25)

Figure 10.3. The effect of endophyte treatment on root fresh weight (g)
(Significant differences apply within species only)



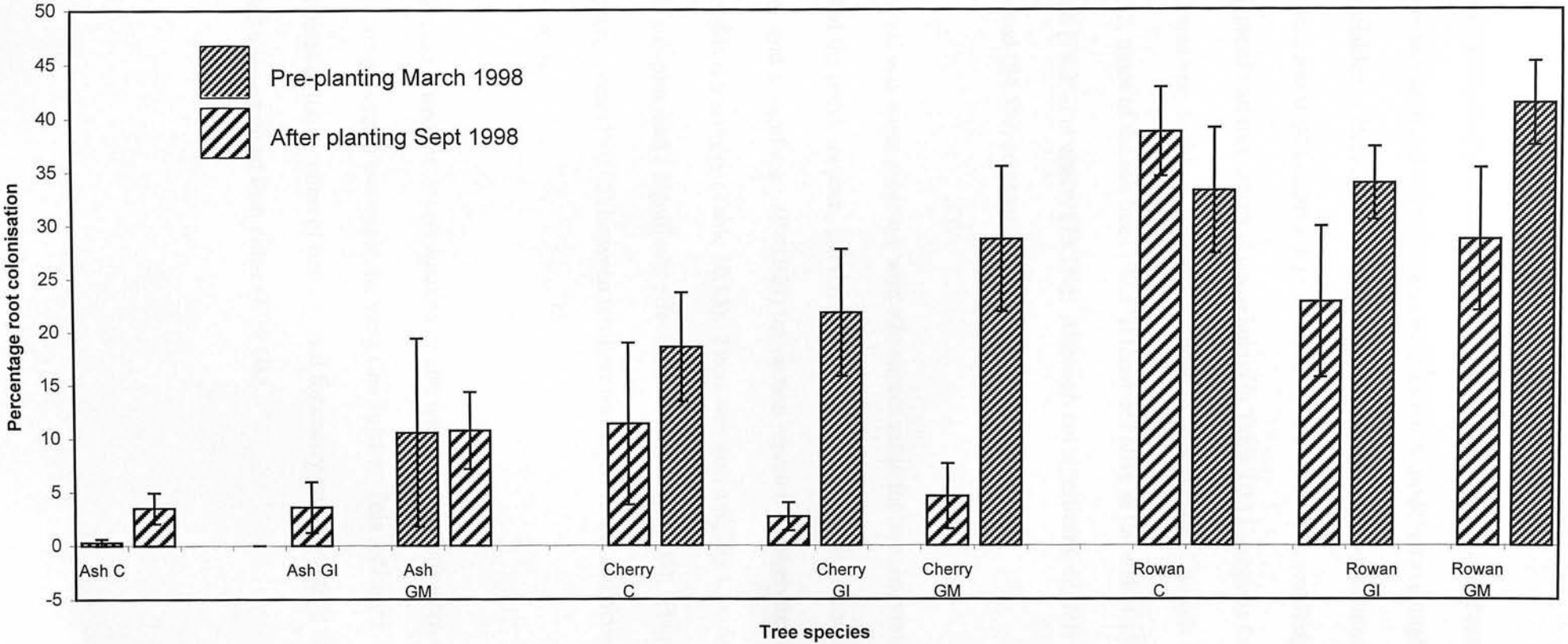
Percentage colonisation

At the end of the first growing season there were no mycorrhizal treatment effects on percentage root colonisation; only tree species was highly significant as a main treatment effect. None of the results were statistically significant, although it appears that the greatest degree of root colonisation occurred in rowan (36%) followed by cherry (23%) and then ash (6%). Across all tree species, the highest degree of root colonisation was associated with the GM treatment (27%) followed by GI (20%) and the control treatment (17%). These are shown below in Table 10.11 and Figure 10.4.

Table 10.11. The effect of tree species and mycorrhizal treatment on percentage root colonisation in 1998.
Figures in brackets indicate standard error of mean and number of replicates.

	Ash	*Cherry*	*Rowan*	Mean
Control	3.5 (1.45, 9)	18.6 (5.11, 9)	33.2 (5.85, 7)	17.2 (3.41, 25)
GI	3.6 (2.38, 9)	21.8 (5.97, 9)	33.9 (4.24, 9)	19.8 (3.47, 27)
GM	10.8 (3.61, 8)	27.7 (6.80, 9)	41.3 (5.08, 9)	27.2 (3.90, 26)
Mean	5.8 (1.56,26)	22.7 (3.41, 27)	36.4 (2.87, 25)	

Figure 10.4. The effect of inoculation on percentage root colonisation before and after planting in 1998.
(Means are significantly different between species only)



Survival

The contribution of the experimental treatment variables to tree survival in September 1998 and May 1999 was assessed using Logistic Regression. A model term comprising the independent variables of trees species, mycorrhizal treatment, fertiliser treatment, and their interactions, was used to assess the probability of survival. The resulting accumulated analysis of variance which is given below in Table 10.11 suggests that tree species ($P<0.001$) was very highly significant as a main treatment effect. As can be seen in Table 10.12, more of the ash trees (90.3%) remained alive at the end of 1998 compared to rowan (78.2%) or cherry (73.1%). Although not significant, slightly more control trees survived (84.5%) compared to GM (81.2%) or GI (77.3%).

In May 1999 survival was again assessed, with allowances made for trees harvested (n=81) at the end of the previous year. Survival was modelled in a similar manner using logistic regression, and a significant ($P<0.001$) interaction reported between tree species and mycorrhizal treatment (Table 10.13). There was also a highly significant ($P<0.01$) effect of sub-plot, and a significant effect of inoculation ($P<0.05$). There were also significant interactions ($P<0.05$) between fertiliser and mycorrhiza, and between species and mycorrhiza.

The effect of treatment * tree species on duration of survival (less than one year, one year, and greater than one year) was explored using Chi-Square. This indicated significant differences in the duration of tree survival for cherry only ($P<0.01$): more of the non-inoculated trees survived than either GI or GM.

Table 10.12. Accumulated analysis of variance to model the contribution of the experimental variables on tree survival in 1998.

	Df	Deviance	Mean deviance	F Statistic
Fertiliser	2	0.0195	0.0098	0.01
Sub-plot	6	14.0570	2.3428	2.34
Tree species	2	20.3937	10.1968	10.20***
Mycorrhiza	2	4.0954	2.0477	2.05
Fert * species	4	4.2775	1.0694	1.07
Fert * myc	4	10.9377	2.7344	2.73
Species * myc	4	8.1162	2.0291	2.03
Fert * species * myc	8	5.2853	0.6607	0.66
Residual	615	576.8784	0.9380	
Total	647	644.0606	0.9955	

Table 10.13. Accumulated analysis of variance to model the contribution of the experimental variables on tree survival in 1999.

	Df	Deviance	Mean deviance	F Statistic
Fertiliser	2	1.246	0.623	0.62
Sub-plot	6	22.926	3.821	3.82**
Tree species	2	39.161	19.580	19.58***
Mycorrhiza	2	7.444	3.722	3.72*
Fert * species	4	3.915	0.979	0.98
Fert * myc	4	14.664	3.666	3.67*
Species * myc	4	9.755	2.439	2.44*
Fert * species * myc	8	1.800	0.225	0.23
Residual	535	674.063	1.260	
Total	567	774.947	1.367	

Tree 'survivorship' at 0, 5 and 13 months is presented below in a life cohort table (Table 10.14) as described in Section 6.6. The variable x refers to age in months; ax refers to the actual number of trees surviving at each time period, and lx the same figure standardised out of 1000 trees. This figure is easily converted to percentage survival by dividing by 10. Log lx refers to 'survivorship', and Kx (the difference between succeeding log lx) may be interpreted as the 'killing factor'.

Table 10.14. Life cohort table for tree survival at planting (0), September 1998 (5 months) and May 1999 (13 months) on Stankards Bing.

X (age in months)	ax	lx	Dx	Qx	Log ax	log lx	Kx
All trees	567	1000			2.757	3.00	
5	439	774	226	0.223	2.642	2.89	0.11
13	332	586	188	0.243	2.521	2.77	0.12
By tree species							
Ash	189	1000			2.276	3.00	
5	168	889	111	0.111	2.225	2.95	0.05
13	135	661	228	0.265	2.130	2.82	0.13
Cherry	189	1000			2.276	3.00	
5	131	693	307	.0307	2.117	2.84	0.16
13	84	444	249	0.359	1.924	2.65	0.19
Rowan	189	1000			2.276	3.00	
5	140	741	259	0.259	2.146	2.87	0.13
13	113	598	143	0.193	2.053	2.78	0.09
By tree species, treatment							
Ash C	63	1000			1.80	3.00	
5	58	921	79	0.079	1.76	2.96	0.04
13	45	714	207	0.225	1.65	2.85	0.11
Ash GI	63	1000			1.80	3.00	
5	55	873	127	0.127	1.74	2.94	0.06
13	49	778	95	0.109	1.96	2.89	0.05
Ash GM	63	1000			1.80	3.00	
5	55	873	127	0.127	1.74	2.94	0.06
13	41	651	222	0.254	1.61	2.81	0.13
Cherry C	63	1000			1.80	3.00	
5	52	825	175	0.175	1.72	2.92	0.08
13	38	603	222	0.279	1.58	2.78	0.14
Cherry GI	63	1000			1.80	3.00	
5	36	571	429	0.429	1.56	2.76	0.24
13	22	349	222	0.389	1.34	2.54	0.22
Cherry GM	63	1000			1.80	3.00	
5	43	682	318	0.318	1.63	2.83	0.27
13	24	381	301	0.441	1.38	2.58	0.25
Rowan C	63	1000			1.80	3.00	
5	45	714	286	0.286	1.65	2.85	0.15
13	40	635	79	0.111	1.60	2.80	0.05
Rowan GI	63	1000			1.80	3.00	
5	49	779	221	0.221	1.69	2.89	0.21
13	34	540	239	0.307	1.53	2.73	0.16
Rowan GM	63	1000			1.80	3.00	
5	46	730	270	0.270	1.66	2.86	0.14
13	39	619	111	0.152	1.59	2.79	0.07

Using the life cohort table, it may be seen that at the first time period (September 1998) the greatest killing power (Kx) by tree species was demonstrated by cherry (0.16), followed by rowan (0.13) and ash (0.05). However, by May 1999 the order is cherry (0.19) followed by ash (0.13) and then rowan (0.09), suggesting that a lower rate of winter kill was experienced by rowan.

When killing power is consider by tree species and mycorrhizal treatment, it is noticeable that across all species, in September 1998, the mycorrhizal treatments all have a higher killing power than the control treatment. By May 1999, there are differences between the species: in ash the lowest killing power is associated with GI (0.05), in cherry with the controls (0.14), and in rowan with the controls (0.05). Differences in survivorship (lx) are shown in Figures 10.5 to 10.7. As noted above, using Chi-squares, the difference in survival by mycorrhizal treatment is significant for cherry only.

Figure 10.5. The effect of inoculation on survivorship (log lx) in ash
(No significant differences between treatments using Chi Squares).

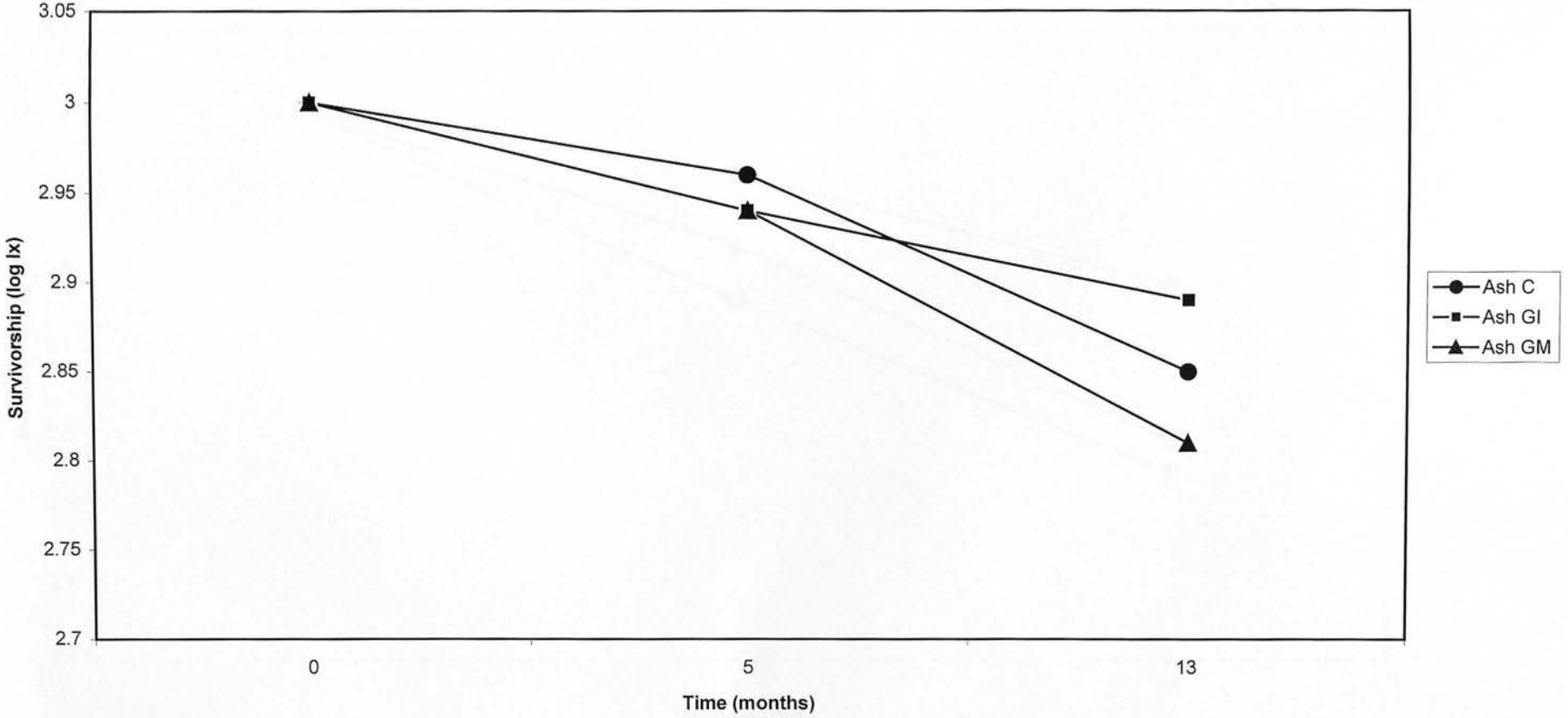


Figure 10.6. The effect of inoculation on survivorship (log lx) in cherry.
(Differences between treatments highly significant (P = 0.001) using Chi Squares)

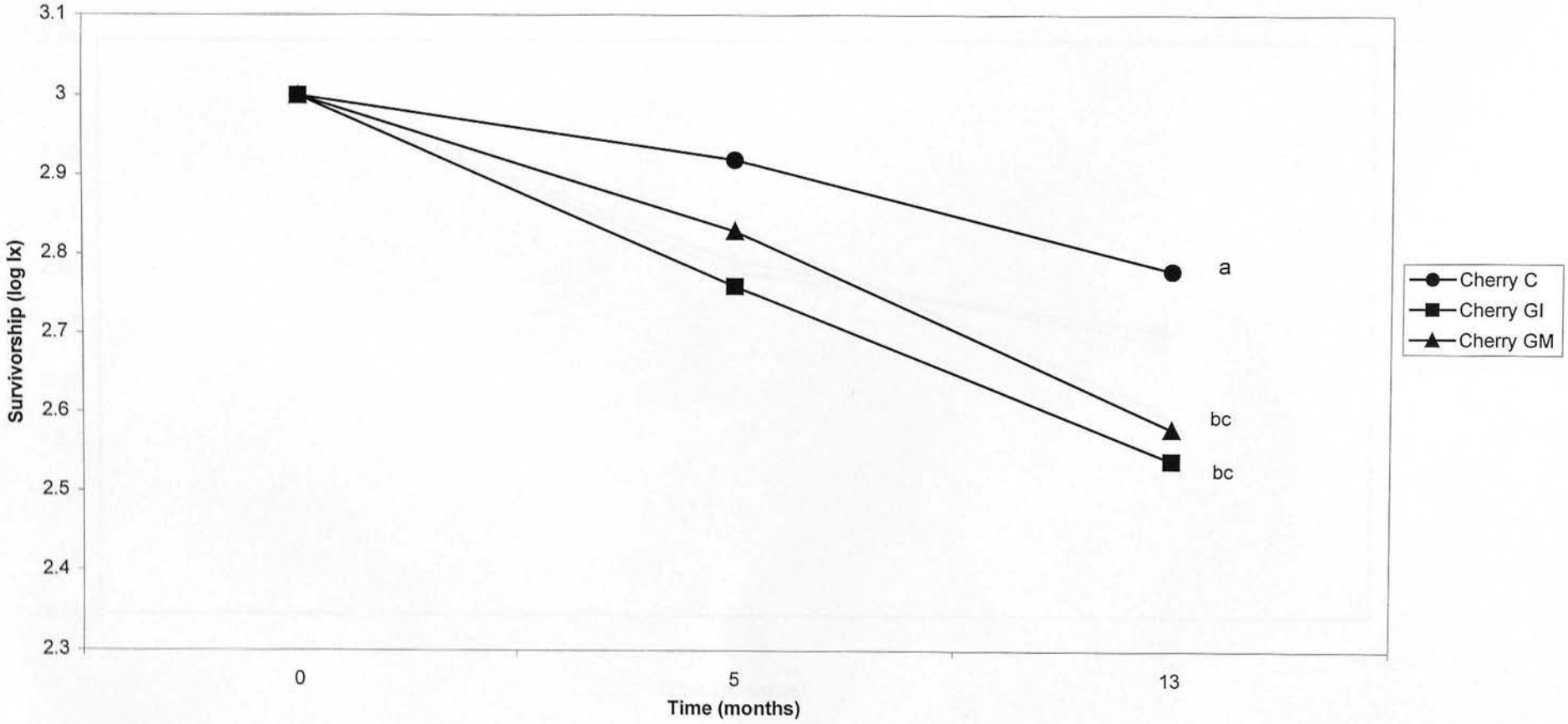


Figure 10.7. The effect of inoculation on survivorship (log lx) in rowan.
(Differences between treatments not significant using Chi Squares)

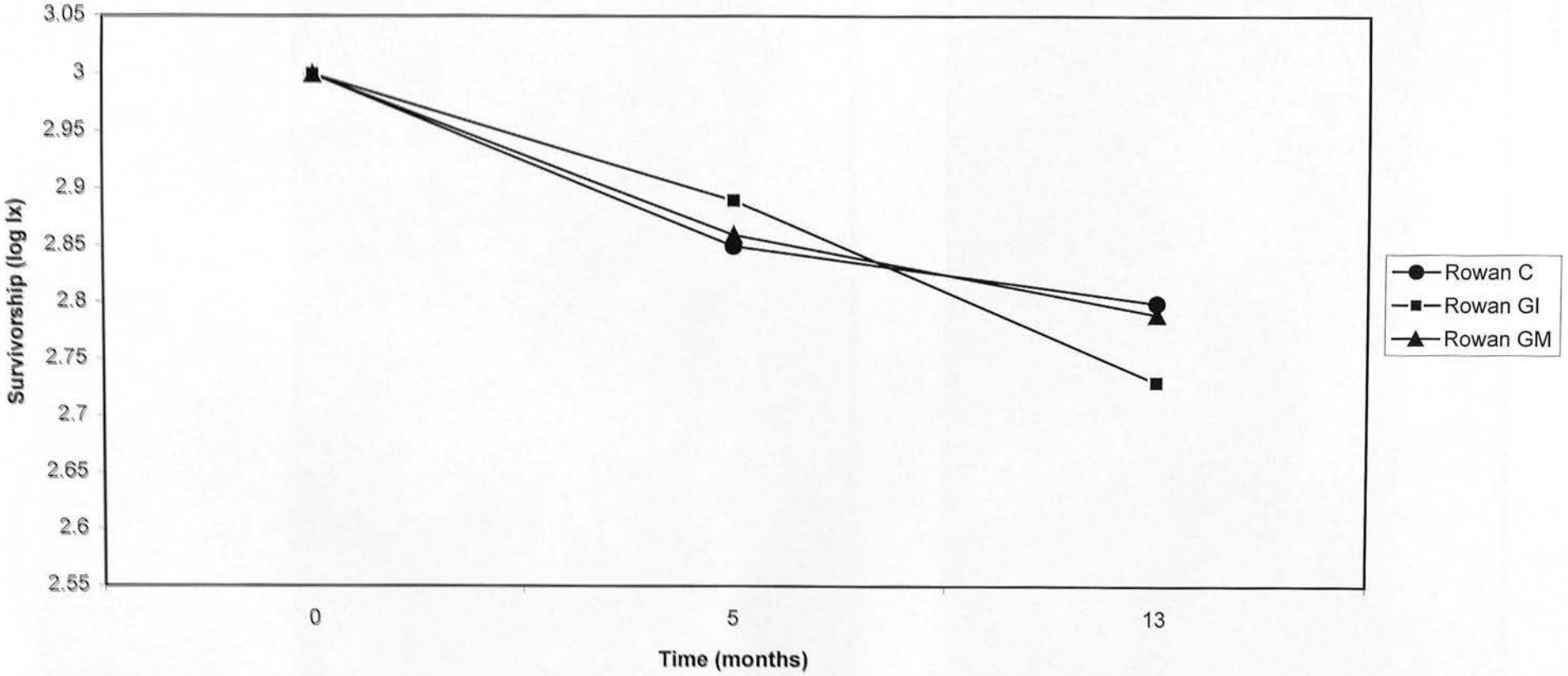


Figure 10.8 Stankards Bing

Figure 10.9 Experimental plants of Ash, Cherry and Rowan prior to planting

ROWAN CHERRY ASH

Control

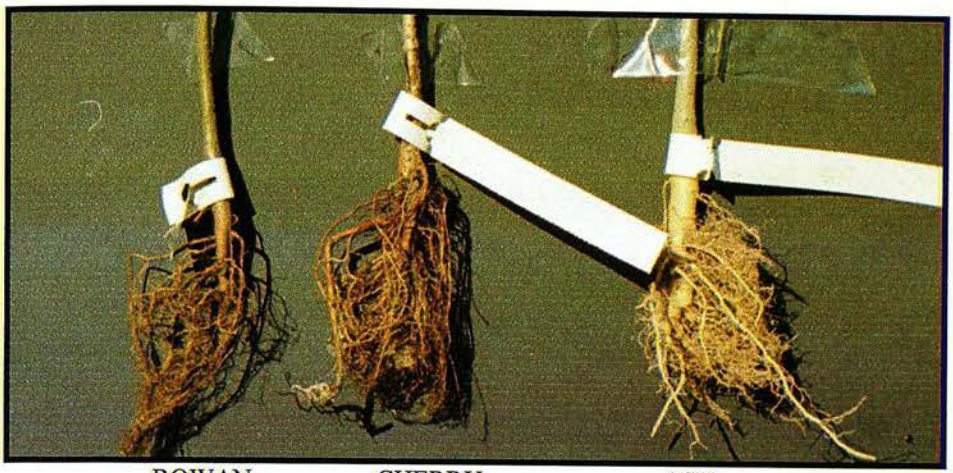
ASH CHERRY ROWAN

G. intradices

ASH CHERRY ROWAN

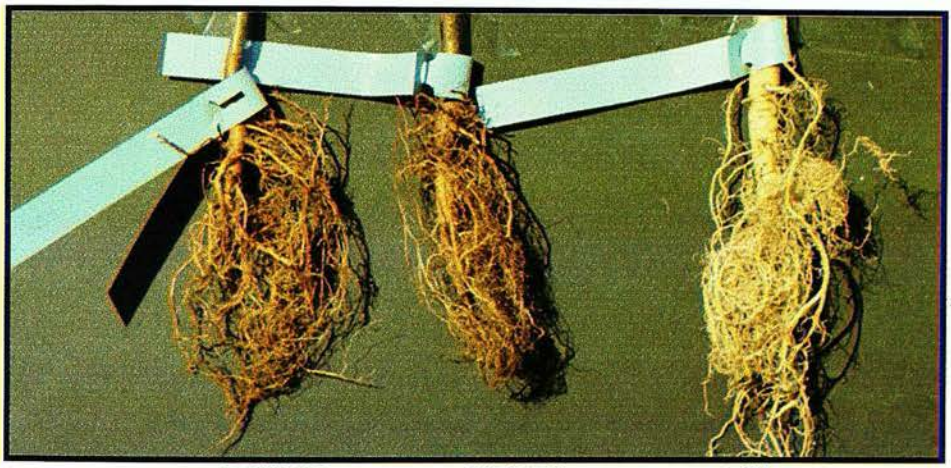
G. mosseae

Figure 10.10 Root systems of experimental plants in September 1998



ROWAN CHERRY ASH

Control



ROWAN CHERRY ASH

G. intradices

ROWAN CHERRY ASH

G. mosseae

10.4. DISCUSSION

10.4.1. Introduction

The aims of this experiment were to consider the effects of inoculation with AMF on the growth and survival of a range of native trees under field conditions, interactions between trees species and mycorrhizal treatments, and also the effects of a management practice such as fertiliser regime. A split-plot experiment was set up comprising mycorrhizal treatment (3 levels); tree species (3 levels) and fertiliser regime (3 levels). Experimental trees were monitored for one year following transplanting. The previous experiment had suggested that AMF affected the growth and survival of rowan under standardized experimental conditions, although a search of the literature revealed that few experiments had been conducted under realistic landscape conditions. The site chosen for the experiment was steeply sloping and exposed, with heavily compacted (bulk density of 1.7 mg m^{-3}), relatively infertile and slightly alkaline (pH 8) soil, comprising mostly building detritus. The site also had a rabbit problem.

10.4.2. Summary of treatment effects.

1. Tree species had a marked effect on tree growth and survival following transplanting. In general the ash trees produced more shoot extension and were in better condition than the other two species at the end of year 1998. At the end of 1998, the cherry trees were nearly three times as likely to die, and rowan more than twice as likely to die, compared to ash. However, by the start of 1999, the rowan trees had the greatest chance of survival compared to the other two species.
2. The mycorrhizal treatment had few effects on growth, with the exception of changes in stem diameter. In year one, survival across all species was reduced in the inoculated treatments. By year two there were interactions between AMF

treatment and tree species. There was also an interactive effect between tree species and mycorrhizal treatment on condition and root fresh weight.

3. Mycorrhizal treatment had no effect on percentage root colonisation, although there was a highly significant effect of tree species: the rowan was more heavily colonised (36%) than either cherry (23%) or ash (6%).
4. Fertiliser had no effect on growth, survival or mycorrhizal colonisation during the first season.

10.4.3. Effects of inoculation on percentage colonisation

There were differences between the tree species in the initial level of root colonisation by indigenous AMF, and also the way in which root colonisation was affected by inoculation. Levels of root colonisation across all treatments increased slightly from April (13%) to September (21%), and although some of this may be attributable to seasonal changes in AMF populations linked to root growth, some may have been due to AMF inoculation. Klironomos [95] and Douds and Chaney [76] both observed a seasonal relationship between root activity in the host and AMF colonisation. In this experiment, across all tree species, colonisation remained at around 17% from April to September in non-inoculated trees, but increased from 8 to 20 % in GI, and from 15 to 27% in GM. The lack of increased colonisation in the control trees may have been associated with the lack of indigenous AMF on site. Although not assessed, it is likely that the site did not have a particularly high population of AMF as it had only been recently re-graded, and was therefore still highly disturbed and poorly vegetated, with the exception of a few weed species.

Root colonisation by AMF was poor in ash: only GM was associated with any significant amount of root colonisation, and this remained at approximately 10%

between the assessments made in April and September. Root colonisation by indigenous fungi tended to be low (less than 5%), and inoculation with GI failed to increase root colonisation. In cherry, root colonisation was low in April (6%), but increased across all treatments by September, and was highest in the GM treatment (28%). In rowan, root colonisation by indigenous AMF was already quite high in April (39% across all treatments), although it was slightly lower in the inoculated trees (23% in GI and 29% in GM). These levels were within the range observed across the range of sites and nurseries sampled in the field study (Chapter 7). By September, root colonisation in the inoculated trees had increased by about 10% in both the GI (34%) and GM (41%) treatment.

Across all tree species the GM treatment was associated with slightly higher levels of root colonisation. These results contrast with those observed in the previous experiment where GM failed to cause any appreciable levels of infection in rowan. It is possible that the GM inoculum used in this experiment was of a higher quality than that used in the previous experiment (not assessed), or that the isolate used was more adapted to the prevailing substrate conditions both in the nursery and on site. AMF adaptation to particular soil and artificial substrate conditions, have been observed by other researchers [135, 136, 75].

It was also interesting to note that root colonisation in ash was comparatively low. The references provided in Harley and Harley [66] suggested that ash formed associations with AMF, and Lovato *et al* [75] recorded 70% infection levels in micro-propagated plants inoculated with GI and grown in a peat-based substrate. Both Kormanik *et al.* [77] and Douds and Chaney [76] found that inoculation improved the growth of *Fraxinus pennsylvanica*, the closely-related green ash, where colonisation levels of 60 to 80%

were observed, and so it is surprising that inoculation had so few effects. Ash also tends to have coarse, fleshy roots, which might be expected to increase host dependency on AMF [105]. One possible suggestion for the low level of colonisation in the present experiment is associated with root phenology. Ash comes into leaf relatively late in the season, and this may be linked with a later commencement of root activity, and hence AMF colonisation. The ash trees were inoculated in February and assessed in April, perhaps before new root growth and therefore infectable roots had commenced. It was also observed that AMF structures were relatively sparse in the coarse roots (diameter >1mm) and more numerous in the finer roots (diameter <1mm). These aspects of root phenology and architecture would appear to merit more attention in future site-based experiments.

10.4.4. Effects of inoculation on growth and survival

There were few effects of inoculation on shoot extension or shoot biomass during the first year of the experiment. Inoculation appeared to have no effect on the number of trees that survived when modeled using logistic regression. However, when the cohort life tables were considered, inoculation appeared to increase the chance of dying during the first year in all species. By the start of the second year, inoculation with both GI and GM continued to reduce the chance of surviving in cherry; in ash GI increased the chance of surviving and in rowan GM and control trees had about the same chance of surviving. Overall, survival was 77% in the first year, and 59% at the start of the second year. The only growth parameters affected by inoculation were changes in stem diameter and condition, which appeared to decrease slightly with inoculation, and root fresh weight, which appeared to increase.

To some extent, the lack of effects on shoot height and biomass may be explained by die-back resulting from a combination of transplanting shock, grazing and mechanical damage, and frost shortly after planting. Mean shoot extension across all treatments was only 6 cm, but ranged from almost total dieback to increases of nearly 40 cm. Shoot extension in the first year following transplanting was less than that observed in similar studies, which used bare-root stock. Gilbertson and Bradshaw [10] recorded 15 cm shoot extension on inner city sites; Kendle *et al.* [9] up to 15 cm (depending on stock source) in good planting conditions, and Hunt *et al* [245]15 cm on a compacted clay loam soil. However Hodge [11], presented data from a road embankment site near Edinburgh, which compared bare-root and cell-grown oak (*Quercus robur*). He noted 6 cm shoot extension in bare-root trees, but 1.6 cm dieback in the cell-grown trees, perhaps owing to a failing of the roots to grow beyond the nursery substrate into the planting notch. The trees used in this experiment were cell-grown.

Stem damage occurred to many of the trees due to rabbit grazing, the fitting of the tree guards, and subsequent abrasion, as well as some die-back due to transplanting, and it was difficult to distinguish the effects of these factors. In addition, some of the cherry, which were already in leaf at the time of planting, suffered frost damage to the soft new growth following snow as few weeks after planting. It is anticipated that in the second and subsequent years, the effects of shoot damage will be reduced, allowing any potential differences due to the experimental, rather than those attributable to site and other factors, to become apparent.

The 'condition' variable was strongly linked with shoot die-back and shoot extension as trees were scored as '4' if there was stem damage and die-back occurred, and '1', if almost complete die-back was reported. In this variable, there were differences

between tree species and mycorrhizal treatment. In both ash and cherry, the inoculated trees appeared to be in slightly worse condition than the control trees; in rowan, while not statistically significant the inoculated treatments were in slightly better condition than the controls. Although much of the reduction in condition and shoot extension may be attributable to mechanical stem damage, it is assumed that this would have been randomly distributed across treatments, whereas die-back due to transplanting stress would not. This may suggest that for condition and stem die-back, inoculation was not beneficial in ash and cherry, but non-deleterious, if not beneficial in rowan.

The changes in stem diameter after one season on site, although slight (less than 0.1 mm) suggested significant but unclear mycorrhizal effects. Only in ash were there increases in stem diameter, the greatest of which was associated with GI (0.2mm); in cherry and rowan, stem diameter appeared to decrease across all treatments, being greatest with GM (-0.3 mm), but least with GI (-0.1 mm). Thus, GI appeared to have the least negative effect on stem diameter across all treatments; GM the most. These decreases were unexpected, and while to some extent may be accounted for by a possible slight change in the position at which the measures were made, this does not explain why only some of the measures (associated with inoculum treatment) appeared to decrease. It is also possible that there were natural differences in bark thickness between March and September, perhaps due to abrasion, or associated with environmental conditions within the tree guards. However none of these explanations would appear to account for within treatment, rather than random differences. A final possibility could be seasonal fluctuations in stem water content between the initial measure in early spring and the final measure in early autumn. It is known that evaporative demands and drought stress can both cause diurnal fluctuations in stem diameter, usually assessed using a dendrometer [239]. Although shoot growth (evidenced

by the formation of a terminal bud) had appeared to cease by September when measurements were taken, many of the trees were still in leaf, and hence transpiring. Is it possible that cherry and rowan, particularly those inoculated with GM, where decreases in stem diameter were observed, were affected by drought stress, but that the ash were less affected? This would appear to indicate possible AMF modifications to host physiology.

Root mass was the only variable positively affected by inoculation. It is worth remarking that root dry mass (corrected for the amount sampled for mycorrhizal assessments) was not significant, but fresh weight of the entire root system was. Assuming that fresh root tissue has the same density as water, then fresh weight gives an approximation of the total volume of a root system. However dry weight is more affected by root fibrosity: a root system composed of fine white roots would have a greater moisture content and therefore lower dry weight than one composed of woody roots. As only fine roots were sampled for mycorrhizal colonisation, this may have removed a greater amount of fine roots and so distorted the final dry weight figure. Inoculation increased the root fresh weight of ash by 60% and rowan by 35%, but reduced root fresh weight in cherry by 70%, compared to the controls. Beneficial effects of inoculation on root biomass were observed in the previous experiment, where GI (but not GM) increased root fresh weight by 37% in the first year and 84% in the second year. Plenchette *et al* [73] reported increases of 240% in apple rootstocks grown in non-sterile soil. Similarly, Hooker *et al.* [42]; Berta *et al.* [43] and Tisserant *et al.* [87] also reported increases in root system size, owing to increased branching, particularly of higher order roots. Improvements to root systems have consequences for water and nutrient uptake, and would therefore aid plant establishment following transplantation. It should, however be noted that in the present experiment when the sample of trees

were lifted, due to the compacted nature of the soil, and the use of cell-grown stock, few roots had grown beyond the planting notch.

10.4.5. Effects of tree species

The effects of tree species were significant across all the parameters of growth, survival and mycorrhizal colonisation. That there were differences in performance between tree species when transplanted to a landscape site, was not an unexpected finding. Indeed the taxa used in this experiment (see Appendix One) were selected to some extent on the basis of contrasting life strategies [39] as well as site preferences [4]. In general, more ash trees survived (90%) than either cherry (73%) or rowan (78%), and those that survived had more shoot extension (8 cm) compared to cherry (4 cm) or rowan 6 cm). The root to shoot ratio was highest in cherry (0.98), then ash (0.83) and lowest in rowan (0.63). However, mycorrhizal colonisation at the end of the first year was lowest in ash (5.8%), then cherry (22.7%) and rowan (36.4%), and so it is unlikely that any of the improved performance criteria reported in ash may be attributable to AMF.

According to Grime *et al.*[166] ash has the established strategy of a competitor, adapted to conditions of high nutrients but low disturbance. It also has a distinct preference for base-rich substrates, and is able to tolerate heavy soils and exposure. The soils on Stankards Bing were alkaline (pH 8) and so some of its superior site performance may be due to a closer match between site conditions and host substrate preferences. In a trial comparing tree root growth in compacted road construction materials, Kristofferson [246] observed that ash had a greater tolerance of compacted subsoil than either *Acer platanoides* or *Tilia vulgaris*. This was attributed to root vigour, despite the greater diameter and hence greater penetrative resistance of the ash roots. Cherry, although usually having a high growth rate and preference for neutral or alkaline soils, is not able

to tolerate exposure, and the sudden cold spell following planting resulted in frost damage and consequent loss of early growth. Rowan has a distinct preference for acidic substrates, and is especially palatable to a range of herbivores, and this may account for some of its relatively poor performance.

Differences in root phenology and structure and subsequent mycorrhizal colonisation, have already been mentioned: bud-break and therefore commencement of root growth was earliest in cherry, followed by rowan, and finally ash. It is therefore suggested that the lower levels of mycorrhizal colonisation reported in ash were associated with a later commencement of root growth, and therefore shorter period during which roots were potentially infectable by AMF. There were also differences in rooting structure: cherry and rowan have similarly fine, fibrous roots, which are heavily pigmented; ash has coarse, fleshy roots. In some way, the root systems of cherry and rowan were more liable to become infected by AMF than cherry. These aspects, as well as the issue of mycorrhizal dependency in the different tree species would merit further studies.

10.4.6. Effects of site factors

The main site factors which affected overall plant performance were rabbit damage, which has already been discussed, and soil compaction. The soil was highly compacted with a bulk density of 1.7 mg m^{-3}. Compaction restricts root growth, and is sometimes associated with additional problems of poor oxygenation and reduced available soil moisture [20]. On this site compaction occurred during the site grading process, and was not alleviated prior to planting by soil cultivations. Nadian *et al.* [93] found that while there were some differences between AMF in their ability to tolerate compaction, no mycorrhizal growth was observed above 1.75 mg m^{-3} owing to the small size of soil pores, and increased ethylene production. It is therefore likely that the compaction

experienced on Stankards Bing reduced new root growth and thus infection sites for AMF, as well as reducing extra-radical mycelial growth. This may in part account for some of the lack of mycorrhizal treatment effects. The soil compaction also made planting difficult, and the plants were poorly stable, particularly when the tree guards were applied.

Fertilizers had no effect on growth, survival or mycorrhizal colonisation during the first year of the experiment. This may in part be explained by the soil conditions (compaction) which inhibited root and mycelial growth. However increased weed growth was observed in the zone of applied fertilizer, and this may have a competitive effect on tree growth in subsequent years.

10.4.7. Comparisons with other field experiments

Evidence from field-based trials where indigenous soil AMF occur is sparse, and tends to vary according to the growth stage at which plants were inoculated, as well as subsequent field conditions. Johnson and Crew [244] observed increased growth and survival in azaleas planted into drought-stressed landscapes. Plenchette *et al.* [73] observed large increases in shoot growth and root volume when inoculated apple seedlings were planted into a P-deficient non-sterile soil. They used young plant material (6 weeks old), inoculated while actively growing, and the field was ploughed and fertilized prior to planting. Thus the material was still at a growth stage where the roots were potentially highly infectable, and planted into ground conditions optimal for root growth. Being still at the seedling stage, much higher relative growth rates were also possible. Mycorrhizal colonisation was not assessed prior to planting in their study, although after 3 months, more mycorrhizal structures, particularly vesicles were observed in the inoculated (83%) compared to control treatments (64%). These levels

were much higher than those observed in the present study, suggesting that in their trial the inoculation technique was successful, and root growing conditions optimal for further AMF colonisation.

Visser *et al* [79] observed increased shoot and root growth in a range of actinorrhizal shrubs, inoculated with AMF and *Frankia* at the seedling stage and out-planted onto amended oil sand tailings. Again, the plants were inoculated at an early, growth stage, which may have improved potential AMF benefits, and the sites cultivated and amended with peat. However it is difficult to separate in this instance the effects of AMF and n-fixing symbionts: the shrubs were all considered highly dependent on both types of root symbionts, and a linear relationship was observed between root nodules and shoot growth.

Both Morrison *et al* [80] and Delisle [81] failed to observed post-planting effects due to inoculation treatment across a range of landscape sites. However, in neither case were the plants subjected to environmentally challenging conditions: in Morrison *et al* [80] the plants were well fertilised, and in Delisle [81] the planting was on previously agricultural or forestry sites. In Morrison *et al.* [80], inoculum was applied using a root dip, and plants were erratically mycorrhizal at that time. After one year on site, colonisation levels were still low (10%) in control plants, and slightly higher in inoculated plants (20%), however growth in inoculated trees was either unaffected or slightly reduced. In Delisle [81] AMF root colonisation was moderate (11-40%) at the time of planting; it was not assessed again after planting.

10.4.8. Improvements to design and suggestions for future experiments

One of the main limitations of this experiment was the lack of apparent mycorrhizal treatment effects. To some extent this may be attributable to the presence of indigenous AMF in the nursery prior to inoculation, and poor substrate compatibility. However it is also possible that the timing and inoculation technique were not optimal. In this experiment the one-year old plants were inoculated 6 weeks prior to planting while plants were still more or less dormant. The trees were also cell-grown, and while this meant little disruption to the root system, only those roots on the outside of the container would have come into direct contact with the inoculum. However, it was anticipated that the inoculum would have been transferred along with the trees to the planting notch. Ideally the trees should have been inoculated at the seedling stage, and grown on for a season before planting. As mentioned before, it had been intended to use inoculated plants from the previous experiment, but as so few survived this was not possible, and it was desirable to begin the experiments that year, without the delay of another season.

Soil physical conditions, particularly compaction was not taken into account in the original design. Although the findings are of interest, at this stage it would have been more of interest to investigate field functioning under conditions where root growth was not so limiting. Expense precluded mechanical soil cultivations, and the size of the experiment (648 trees) meant that was not practicable at the time to hand cultivate around the area of each of the trees.

The experiment also highlighted differences in mycorrhizal colonisation between the different taxa, and it would worth investigating whether this was due to variation in root form (diameter and degree of fibrosity) as well as the timing of root growth. It would

also be interesting to consider host dependency and site adaptations: was the superior performance of ash due to the lower level of colonisation and hence carbon cost? Alternatively, were the ash trees better at resisting infection by AMF, or better adapted to the prevailing site conditions? Along with the other experiments in this thesis, it will be worth monitoring the trial over at least one additional season. Finally there would be some merit in studying the functioning of the symbiosis under a wider range of landscape site conditions. Kendle (1996) suggested that on some sites plants may be too stressed to form AMF. It would therefore be interesting to explore the nature and extent of site stress factors that may be addressed by AMF.

10.4.9. Conclusions

In general, this trial has demonstrated few benefits of inoculation with AMF in terms of improved early growth or survival across ash, cherry or rowan. After one season there appeared to be a slightly increased chance of death following inoculation. The only parameter that increased, was root fresh weight, although this was only observed in ash and rowan. There were differences between the endophytes: GM was associated with higher levels of root colonisation than GI. It would appear that for maximum AMF effects, inoculation should take place as early as possible in the production stage when root are at their most infectable and, due to the smaller size of the root system, good overall contact may be achieved between host root and AMF. However, it has yet to be determined whether the effects of AMF persist beyond the early growth stage, as has been suggested by Mason and Wilson [129] and also Lovato *et al.* [75]. It is also apparent that the association seems to function best in conditions of some nutrient deficiency or even drought, providing that soil physical conditions affecting root and mycelial growth are not limiting.

If we return to the null hypotheses proposed at the start of the experiment, then after one season:

- $H_0$4: All AMF species are equally effective endophytes of rowan: reject
- $H_0$5: There are no interactions between AMF and host genotype (tree species): reject
- $H_0$6: Environmental conditions have no effect on the functioning of AMF: reject.

CHAPTER ELEVEN
DISCUSSION

11.1. INTRODUCTION

In this final chapter, the results of the experiments, which relate to the hypotheses set out at the start of the thesis are drawn together, summarised and compared with those found by previous researchers in this subject area. The practical implications of the work, with respect to improving plant quality and thereby plant survival on landscape sites, are then discussed. This is followed by a critical appraisal of the research methodology used in the study, and suggestions for future research.

11.2. SUMMARY OF FINDINGS

The main findings of the study in relation to the hypotheses set up at the start of the experimental section are summarised below in Table 11.1

11.3. RESEARCH CONTEXT

At the start of this thesis, the theoretical literature indicated that the response of rowan to AMF was uncertain, and that despite several decades of 'blue skies' research, the use of AMF was not a fully workable technology on commercial nurseries or landscape sites in the UK. Since then, the situation has changed in a commercial context, with the launch in 1998 of a range of mycorrhizal products from a large and high profile US company. However, there remains a paucity of published scientific trials that demonstrate unequivocally the benefits of inoculation in a field context.

Table 11.1. Summary of main findings.

Hypothesis	Results
($H_0$1)	Rowan does not form AMF : reject • Rowan does form AMF • Typical colonisation levels 29-40%
($H_0$2)	Soil factors have no effect on early growth or survival of rowan : reject • Soil type affects the growth and survival of rowan • Heat-sterilisation reduced competing organisms in agricultural soil; in more 'natural' soils, improved growth associated with biological soil factors such as AMF and other rhizosphere organisms
($H_0$3)	Inoculation has no effect on early growth and survival of rowan: reject: • Inoculation with GI lead to significant (53%) root colonisation under experimental conditions • Associated with increased height (X2) and root mass (X2) after 2 seasons, and increased winter survival (X4) • A higher initial dosage of inoculum associated with greatest root colonisation in first season; irrelevant by second season
($H_0$4)	All AMF are equally efficient endophytes of rowan: reject • GI performed best in sand cultures (Expt.2); GM in field (Expt.3) • In sand, GM failed to colonise roots, associated with reduction in growth and survival compared to controls. • Unclear whether poor quality inoculum, or host-substrate-endophyte preference. • In field, GM associated with highest root colonisation in all tree species: ash (11%); cherry (28%), rowan (41%), but effects on growth and survival mediated by host genotype (tree species)
($H_0$5)	No interactions between AMF and host genotype. Seed provenance: accept; tree species: reject. • Few interactions between with AMF and provenance (Expt 2) • Tree species differed in field performance: ash>rowan>cherry (Expt.3). • Tree species varied in susceptibility to infection: rowan>cherry>ash (Expt.3). • Interaction between tree species and AMF for survivorship (Expt.3).
($H_0$6)	No effects of environmental factors (field conditions) on AMF: reject. • Inoculation increased root colonisation, being higher in GM than GI • Few effects on growth, although root mass increased in ash and rowan • Survival compromised by inoculation in cherry • Species adaptation to site conditions more significant than AMF • Fertilisers had no effect after one season, although increased weed growth may be deleterious in future years.

Field Study

The majority of references cited in Harley and Harley [66] suggested that rowan formed associations with AMF. Exceptions included Trappe [189], in the Pacific North-West, where rowan is not indigenous, and Dominik [180] in Poland, which suggested that ECM associations were sometimes formed. The present study has confirmed that on nurseries and a selection of sites, rowan associates with AMF. Furthermore, when rowan is grown in non-sterile soil, collected from sites where rowan is growing, AMF infections are also formed. No evidence was found of ectomycorrhizal associations.

Experiment One

Inoculation studies using rowan included Vosatka *et al* [115], who observed a loose relationship between AMF colonisation and shoot growth on polluted sites in Slovenia, and Morrison *et al.* [80], who suggested that under nursery conditions the association may be commensal. The first experiment of the present study has indicated that on 'natural' soils, inoculation with indigenous AMF was most beneficial under less fertile conditions. This appears to reinforce the findings of Morrison *et al* [80] that in more favourable nutrient conditions the association may be commensal.

Experiment Two

In the second experiment, substrate and nutrient levels were standardized, in order that nutritional and mycorrhizal effects could be distinguished. Under these conditions, when inoculation was successful and a significant level of root colonisation achieved (over 50%), there was a significant effect on height, root and shoot biomass, as well as an unexpected improvement in winter survival. McKay and McEvoy [141] reported that rowan roots were hardy down to -6°C on a research nursery near Edinburgh (seed provenance not given). In the present study seedlings experienced cold to -18 °C, and

there was a significant difference in survival between seedlings which were successfully inoculated with GI, compared to non-inoculated controls, or those where the inoculation treatment was unsuccessful. Surviving seedlings were larger, and it was not possible to ascertain whether the increased hardiness and stress tolerance was associated with size, or some AMF-mediated change in host physiology resulting in increased hardiness. These effects would have a useful application on exposed sites. However the findings of this study contrasts with Visser *et al* [79] who found that inoculated actinorrhizal shrubs had a higher level of winter kill, which they attributed to changes in nutrition/host physiology which adversely affected cold-hardiness. This aspect (cold-hardiness) would require further studies before unequivocal recommendations could be made to nurseries and landscape practitioners.

Experiment Three

The field experiment attempted to address the lack of published trials of landscape performance in inoculated plants on a hostile site, and expand the relevance by including a range of plant taxa and a management practice, namely fertiliser application. As highlighted in the literature review, few published studies to date have been conducted either on nurseries or landscapes using conditions and techniques similar to those with which practitioners would be familiar. Plenchette *et al* [73] used material less than 6 cm tall, on well-cultivated soils. The magnitude of effects suggested by the work of Kormanik *et al* [77] - in some cases biomass increased by a factor of X80 - suggested either that the range of taxa used were extremely dependent on AMF, or that some other factor may have inhibited the growth of non-inoculated tree seedlings. As previously noted, the work of Morrison *et al* [80] was conducted under well-fertilized and irrigated nursery and site conditions, which would not be expected to challenge plant growth.

In rowan, inoculation initially decreased colonisation compared to non-inoculated plants, which had levels of nearly 40 per cent from AMF indigenous to the nursery at the start of the season. By the end of the season plants inoculated with GM had levels of colonisation nearly 10 per cent higher than the control plants. However the only parameter significantly affected by inoculation was root fresh mass, which increased in ash (+75%) and rowan (+42%), but decreased in cherry (-48%). Increases in root fresh mass were not unexpected: both Plenchette *et al* [80] and Morin *et al* [74] reported increases of over 200% in root biomass following inoculation in apple. Studies such as those by Hooker *et al* [42] suggest that inoculation with AMF is associated with increased root branching, particularly of higher order lateral roots. In the inoculation experiment (Experiment Two) root mass increased by 36% compared to control plants during the first year, and over 180% in the second year. Thus, increased root mass for ash and rowan, but not cherry are in accordance with the findings from similar studies.

In ash, root colonisation by indigenous AMF or GI, was particularly low, being less than 1 per cent. However, inoculation with GM increased root colonisation to 11 per cent, and was associated with increased root mass. The cherry were erratically mycorrhizal at the start of the season, and although root colonisation increased by nearly 20 per cent in inoculated plants, this was also associated with a slight decrease in root mass. Thus, the study indicated that although inoculation with a suitable species of endophyte increased root colonisation, after the first season findings were inconclusive as to whether this was actually beneficial or not, given the site conditions and type of plant material used.

11.4. PRACTICAL IMPLICATIONS

The present study was conducted within the context of poor tree survival on landscape sites. It aimed to explore the way in which this problem might be addressed by an inexpensive and sustainable form of biotechnology. In particular, a decision was made to focus on improving tree quality in the nursery in order to improve plant survival on site, and excluded other anthropomorphic factors implicated in plant handling.

Plant quality implies that the plant is capable of fulfilling the purpose for which it was grown. From the nurseryman's perspective the plant must be of saleable condition and competitive in price. At present regulations cover size ratios, and freedom from negative attributes such as disease or physical damage. Physiological attributes, reflecting potential stock vitality may be assessed, however as yet these attributes are not underwritten by legally binding regulations. In particular attributes such as seed provenance, which can determine growth rate and stock adaptation to site growing conditions, need not be guaranteed for amenity plantings. The recent move by the Horticultural Trades Association to certify plant quality did not include this criterion, which lead to at least one major tree producer withdrawing from membership. Similarly, there is no perceived requirement to verify the presence of mycorrhizas and other root symbionts, despite evidence from many researchers that these organisms are integral to a functioning of a healthy root system.

The present study has furnished evidence that inoculating rowan at an early stage lead to larger plants, with a greater root mass and enhanced stress (cold/desiccation) tolerance, under low nutrient experimental conditions. This would appear to suggest, at the very least that inoculating seedlings which respond to AMF in the nursery may enable larger plants to be grown for a lower given level of nutrients. This could lead to

shorter production cycles, although as tree planting tends to be seasonal, this would not necessarily be of benefit in younger stock. In addition, larger plants are not always best: in some inaccessible forestry plantings, where plants need to be carried to the planting site, larger plants are heavier and therefore more expensive to transport. However on many landscape sites, larger plants give more immediate impact and so might be preferred. Reducing fertiliser inputs, although financially insignificant at present, may become more significant with legislation covering potential fertiliser run-off into water-courses. Thus, it might be argued from the nurseries perspective that, providing the technology is cost-effective, there may be some benefits to be derived from inoculation, at least from the morphological quality of the finished plant.

It is of more interest perhaps, to consider whether there are any additional intrinsic advantages to be gained from selling inoculated plants. That is, can mycorrhizal plants be marketed as 'added-value' products and thus command a premium price that takes account of any costs incurred by inoculation? Are mycorrhizal plants in any way fitter and better adapted to landscape site conditions? Perhaps more pertinently, would landscape practitioners be prepared to pay more for them? The bottom line must be whether a mycorrhizal plant performs any better on site compared to a non-mycorrhizal plant. One frequently used line of argument is that many sites lack AMF, and that nursery practices tend to reduce or eliminate indigenous AMF populations. Even where indigenous AMF are present, these may not be the most effective or efficient at improving the growth and site tolerances of the host plant.

The present study has indicated that non-inoculated rowan seedlings from nurseries, whether cell-grown (Chapter 10), produced in mineral seed-beds (Chapter 7), or grown in a non-sterile soil mix (Chapter 8), have a natural level of root colonisation by AMF

of around 30-40%. Only the seedlings grown in sterile sand or inoculated with an ineffective/poor quality inoculum (Experiment Two) had a low level of AMF present in the root. For the cell-grown plants (Experiment Three) infection levels were around 30% in cherry, but less than 1% in ash. These findings would appear to suggest that providing the growth substrate had not been disinfected, and that the plants were grown outdoors, then in rowan, and probably cherry, plants leaving the nursery would have an adequate level of root colonisation by indigenous AMF. Only the ash seedlings appeared to be lacking indigenous AMF at the time of leaving the nursery.

When landscape sites were considered (the field study), the small sample assessed suggested that given time, most plants would become mycorrhizal eventually. After five years, the levels of root colonisation in rowan trees grown on the reclaimed coal bing and the farm woodland site were broadly similar. On the reclaimed oil-shale bing (Experiment Three), plants that were not mycorrhizal at the time of planting, did not become so after the first season. This suggests either that indigenous AMF were not present in any great number on the site, or that soil conditions, especially compaction inhibited their ability to infect plant roots. Thus on this site, if the plants were not mycorrhizal at the time of planting, they had very little chance of becoming so in the immediate future.

The next factor to be considered is whether root colonisation by AMF (indigenous or applied) actually mattered in terms of improved survival or growth. In the field experiment, inoculation increased root mass but not survival, which tended to be a characteristic of tree species. Due to rabbit damage, it was not possible to detect differences between treatments in shoot extension or die-back. Increased root mass and therefore root volume would be expected to benefit plant establishment, particularly if

it was associated with improved root regeneration and therefore nutrient and water uptake. However after one year on site, there was some evidence that tree survival, particularly in cherry when planted onto a hostile site might be compromised by inoculation.

A further implication of this study is whether plants should be inoculated for nursery growth or field performance. As we have seen, nursery performance in rowan (chapter 9) was enhanced by inoculation with GI; under field conditions (Chapter 10) GM appeared to be the most effective endophyte. Nursery cultural practices and environmental conditions are usually optimised to produce a saleable plant; site conditions are rarely optimal. Is there any scope in growing plants in conditions more analogous to those in which they will later have to perform, assuming that this is known at the production stage? While this may seem anathema to many nurseries, after all how do you persuade a plant buyer that smaller but fitter is a desired plant attribute, without calling to mind similarities with the emperors' new clothes? Most mycorrhizal scientists agree that reducing nutrient and irrigation regimes encourages plant dependence on AMF. However precise, practical guidelines for the degree of stress permissible before plant survival is adversely compromised have yet to be determined, bearing in mind the carbon cost to the plant of AMF formation and upkeep.

The cost of inoculation in relation to expected benefits also have to be included in the decision-making process. In this experiment inoculation would have cost around 4p per plant, against a total price of 20-30p for a cell-grown tree at the time the study was carried out. Thus from the nurseries perspective, inoculation may not be considered worthwhile. However if inoculation had been carried out at an earlier growth stage

using compost incorporation, costs might have been as low as 1p per plant, which may have seemed a more attractive option. Whether a landscape architect might be persuaded to pay a premium for an inoculated plant would depend on a greater likelihood that more plants survive and therefore replanting costs can be reduced.

Further practical implications are concerned with issues of quality control of the inoculum, and verifying that plants have actually been inoculated. Assessing the presence of AMF is time-consuming and requires basic laboratory facilities and staff skills, which may be beyond the means and expertise of many nurseries. Thus if plants are to be marketed as mycorrhizal, there will be a demand for laboratories offering these facilities. There has been some concern expressed that many products currently on the market may be no more than 'snake-oil' (St John, pers. comm.). The experiments carried out in this study indicate that inoculation is not always successful, and while there may be some indication of host-substrate-AMF incompatibility, it cannot be ruled out that some products may not contain viable AMF propagules. At present no industry standards exist to control the quality of inoculum.

11.5. METHODOLOGY

Limitations of the research methodology were considered in each of the experimental chapters, however some points are common to the series of experiments and merit further discussion. These include: the appropriateness of the chosen growth parameters; exploration of the mechanisms underlying treatment effects, and technical issues associated with inoculation techniques and site preparation.

Were the data measures the most appropriate indicators of plant quality and therefore predictors of field performance? Across all the experiments, reliance was placed on morphological attributes (stem height and diameter, leaf number, root and shoot biomass). These are cheap and easy to assess, and widely used in practice: most plant grading is dependent on size. While to some extent size may reflect carbohydrate reserves and therefore growth potential – current shoot extension usually correlates well with previous shoot extension - there is some concern that size may not relate to plant vitality or physiology. Also, some of the AMF effects on host physiology may have been more qualitative than quantitative, for example changes in carbohydrate allocation and nutrient status, modifications to rooting structure, host phenology and cold-hardiness. A greater exploration of these areas would have yielded more information on the mechanisms underlying AMF functioning.

Root colonisation data by AMF was assessed, usually seasonally as an indicator that some symbiotic association had been formed. More frequent assessments would have enabled the pattern of infection to be plotted with greater accuracy. Percentage root colonisation data also has a large variance, and rarely correlates with growth enhancements. Many researchers prefer to break down the components of colonisation into vesicles, arbuscules and hyphal coils, to give an indication of AMF functioning: a higher proportion of arbuscules suggests that the association is more metabolically active. Others have combined root colonisation data with assessments of AMF physiology, for example production of alkaline phosphatase or succinate. There would also have been merit in considering the AMF species composition and infectivity of the soils and inoculum used in the experiments. Identification of the species which associate naturally with rowan, would have given an indication of suitable AMF isolates for inoculation, as well as information on their ecological preferences. Both

the inoculation experiments suggested host-endophyte-substrate preferences, but neither the identity nor viability of the inoculum used was verified at the start, which later emerged as a significant omission. Assessments of indigenous AMF present on the field site prior to planting, how this was modified by the introduction of inoculum on the plant roots, and whether the inoculum persisted under field conditions would also have been useful.

Finally, there are technical issues concerning the inoculation technique, and ground preparation of the planting site. The first inoculation experiment (Chapter 9) which used a sterile and neutral growth substrate demonstrated that inoculation with a suitable endophyte lead to dramatic growth increases. Inoculum was successfully incorporated into the substrate at an early growth stage of the plant. However it was not feasible to repeat the process for the field experiment (Chapter 10) and doubt remains as to the effectiveness of the inoculation technique. The plants were inoculated at a later growth stage, using a substrate not tested for its receptivity to AMF, and insufficient time may have elapsed for the symbiosis to become established prior to planting. At the time of planting, no ground preparation of the site was undertaken, mainly for practical and financial reasons, and perhaps also reflecting commercial practice! Compaction later emerged as a significant factor inhibiting both root and mycelial growth, and despite the widespread occurrence of this problem on landscape sites, field functioning rather than compaction was the hypothesis under consideration. Some alleviation of compaction, might have given a more conclusive indication of the benefits of inoculation to practitioners.

11.6. FUTURE WORK

Potential areas for future investigation include the phenomenon of cold tolerance, the role of indigenous AMF, and expansion of the work to consider a wider scenario of site factors and host dependency, and thus arrive at a more reliable landscape management technique. The enhanced winter survival of rowan following inoculation with a suitable endophyte was an unexpected finding. However it was uncertain whether this might be attributable to plant size – the bigger and perhaps more developmentally mature plants tended to have a greater chance of surviving, or some AMF-induced change in host physiology which affected cold-tolerance. As the findings of this experiment were in conflict with those encountered in other studies, and the potentially disastrous consequences of false recommendations, this aspect would merit closer examination.

The role of indigenous AMF was implied by the soil experiment (Chapter 8). A greater exploration of the potential for indigenous AMF to affect host growth, and the extent to which they made be modified by soil factors, management practices and inoculation with 'exotic' species of AMF, would be of interest. In particular, the potential use of non-sterile soil as an inoculum (already practiced by a major producer of cell-grown trees), and the modification of cultural and management practices to enhance natural populations of indigenous AMF.

While the present study has not provided clear-cut evidence of AMF benefits under field conditions, there is a need to identify precisely which combination of host plants and site factors may be addressed by AMF. The issue of host dependency / susceptibility to root colonisation was suggested in ash. There is some potential in coinciding inoculation with the onset of root growth and therefore the production of

young, infectable roots. Other cultural/environmental and genetic factors that govern host plant responsiveness to AMF need to be clarified.

Finally there are the practical issues of technology transfer: how to demonstrate unequivocal AMF benefits, address issues of inoculum quality control, arrive at precise cultural and management guidelines, and most importantly persuade nurseries and practitioners to adopt the technology.

11.7. GENERAL CONCLUSIONS

Returning to the question posed at the start of this thesis: how *is* tree quality in rowan affected by mycorrhizal fungi? Rowan does form AMF, and when grown on soils collected from sites where rowan occurs naturally, biological factors which include AMF, appear to enhance its early grown and survival. Under experimental conditions, inoculation with a suitable endophyte, which leads to a significant amount of root colonisation, is associated with a bigger plant that has an enhanced ability to tolerate winter freezing and desiccation. Thus, AMF do appear to improve plant quality in a morphological and perhaps physiological context at the nursery stage. However, the acid test of plant quality – that of any potential carry-over effects of inoculation into field performance – remains unclear. To this end a conceptual mycorrhizal decision model is presented in the next chapter, that may be amended as new research results become available. It is also suggested that future definitions of tree quality are enlarged to include the concept of innate genetic and ecological fitness to purpose, implying the significance of selecting both plant and rhizosphere organisms such as AMF on the basis of adaptation to final site conditions.

CHAPTER TWELVE.

TOWARDS A MYCORRHIZAL DECISION MODEL FOR LANDSCAPE MANAGEMENT.

12.1. INTRODUCTION

Plant establishment may be difficult on some urban landscape sites due to a combination of poor initial plant quality, hostile site conditions, lack of suitable aftercare and vandalism. The literature review and experimental section have provided some evidence that AMF may be incorporated at the nursery stage to improve plant quality. Assuming that environmental and cultural conditions are favorable to the formation of mycorrhizas, plants inoculated with suitable AMF tend to be larger and have a greater root mass, compared to non-mycorrhizal plants, particularly at reduced nutrient levels. There is also some evidence that AMF plants have an increased tolerance of some environmental stresses, for example the cold and drought tolerance of rowan observed in Experiment Two. However, does inoculation with AMF confer post-transplanting benefits, in excess of those associated with improved early growth? And if so, in what planting situations is it likely to be the most cost-effective solution to improving plant performance?

This study, which comprises a mixture of literature, experimental results, informal telephone interviews/questionnaires, and decision theory, has been undertaken to develop a conceptual model towards identifying:

1. Environmental conditions in which AMF might be effective in improving plant growth
2. Landscape scenarios in which AMF may provide an economically viable approach to improving landscape tree performance.

It is anticipated that the resulting model may be tested as more research information becomes available.

Telephone interviews and questionnaires were sent out to a sample of landscape architects from the Scottish Chapter of the Landscape Institute (n=20); Scottish native tree nurseries (n=10) and mycorrhizal scientists (n=10) as part of a pilot study, in May 1997. There were too few responses for valid statistical analysis, and so responses are only treated qualitatively throughout the text.

12.2. APPROACHES TO THE PROBLEM

There is a lack of clear and comprehensive findings from field trials using a sufficiently broad range of plant taxa, environmental and management factors to simulate the situations likely to be encountered by landscape practitioners. Yet with the current commercial interest in mycorrhizas, AMF are being marketed as a low-cost insurance strategy to reduce transplant losses, with scant attention given to amending landscape specifications, or the risk that AMF may actually compromise plant survival. Against this background there is a need to predict the likely response of landscape plants to inoculation with AMF. Towards this end several approaches to the decision-making process are suggested. These encompass a basic physiological approach based on plant-fungus carbon economy; an economic model which uses the cost-benefits in terms of increased plant yields against management/inoculation costs, and a combined economic-physiological model, more appropriate to a landscape scenario where 'benefits' may equate to a more subjective appraisal of plant performance.

12.2.1. *Physiological cost-benefits model.*

Fitter [238] proposed a simulation model based on increased carbon fixation by the plant, attributable to increased phosphate influx into the root as a direct result of mycorrhizal infection. His model assumed that P-uptake was the most important benefit to the plant, which could be related to increased plant fitness, and allowed for differences between soils in P buffer power and moisture concentration. He cited evidence that for onion roots growing in a sandy loam soil, the 'zero sink' rate, which must be exceeded if mycorrhizal assistance with P uptake is invoked, was 3.5 pmol m^{-1} root length s^{-1}. In other words, above this threshold P demand exceeded the ability of the root system to supply it, and below this level P demand was too low to justify the cost of maintaining the mycorrhizal association. Under natural conditions, plants may not always experience peak P demands necessitating mycorrhizal uptake, and would therefore vary in their need for mycorrhizal infection.

Fitter then related the number of mycorrhizal entry points (NEP) in the root system to the P flux achievable through each entry point, and suggested an optimal NEP where carbon benefit (C_{in}) was equal to carbon cost (C_{out}). The carbon cost or 'sugar tax' to the plant is usually around 10 per cent of carbon transported to the root system, and is used for growth and maintenance of the mycorrhizal fungus. Depending on the P inflow value of non-mycorrhizal roots, and the cost and efficiency of the fungus, there would be an optimal P inflow value above which the plant was more likely to be non-mycorrhizal. Thus, Fitter's model depended on the ability of the soil to supply P, and the ability of the root system to meet the plants' P requirement at any particular time, against the cost of maintaining the fungus, to predict whether the association was likely to be of benefit.

12.2.2. *Economic cost-benefits model.*

Miller *et al* [124] proposed an economic model to evaluate the potential benefits from inoculation in an agricultural or managed ecosystem context. Again, assuming that the major advantages to be gained from inoculation were concerned with improved yield attributable to phosphate uptake, they proposed:

> Net Benefit (NB) = $(MNR_{m+} - MNR_{m-}) - MC + EB$
>
> Where:
>
> MNR_{m+} = management net return with mycorrhizal management. This is the difference between total costs and revenue.
>
> MNR_{m-} = management net return without mycorrhizal management
>
> MC = cost of management of mycorrhizas
>
> EB = environmental benefit to society/farmer, where applicable

Three possible response patterns were suggested:

i) Increased P uptake, where P supply is limited due to fertilizer unavailability or lack of money. This would be more applicable in a developing country rather than an urban landscape context – fertilizer is usually readily available and is a small proportion of total planting costs (see Section 12.3.4).

ii) Increased P uptake and yield at low P levels with mycorrhizal management (M+), but increased yield with higher levels of P uptake without mycorrhizal management (M-). This response, as in the previous assumes that P uptake is the major factor limiting plant growth, but that it is more cost-effective to apply fertilizer than use M+ (Figure 12.1).

iii) Increased yield with m+ due to benefits in addition to P uptake, for example increased uptake of other limiting nutrients, improved tolerance of soil toxicity, reduction in damage from pests and disease, effects on hormonal relations or improved plant-water relations (Figure 12.2). This response is probably more applicable to landscape sites, where according to Bradshaw *et al.* [12] drought is often the most common chronic factor inhibiting plant growth.

Limitations to this model in a landscape context include translating yield, with its easily defined economic value into an acceptable definition of plant performance, identifying the factors restricting plant performance, and assessing the likelihood of benefits. Therefore, a third approach is proposed.

Figure 12.1. Generalised response curve where maximum revenue achieved by increased P application (M^-). (Source: Miller *et al.*[124]).

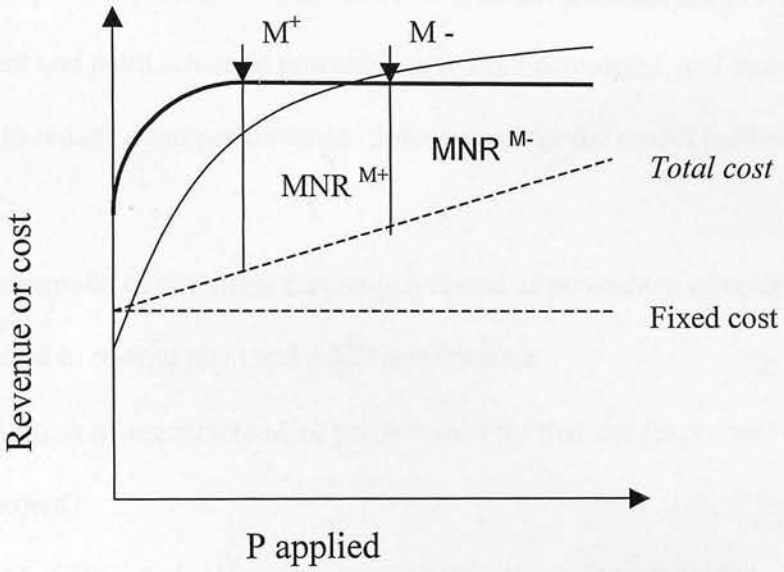
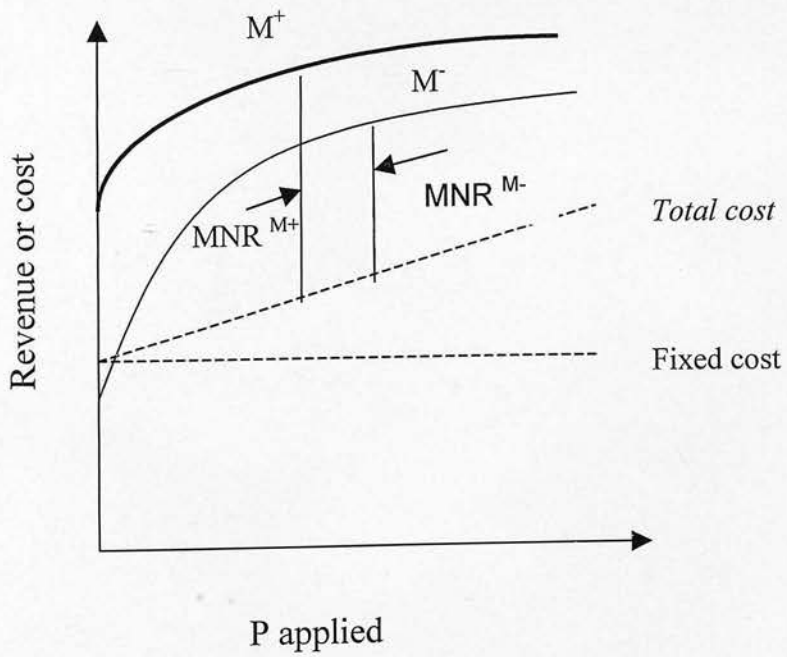


Figure 12.2. Generalised response curve where additional benefits to be gained from mycorrhizal management (M^+). (Source: Miller *et al.*[124]).



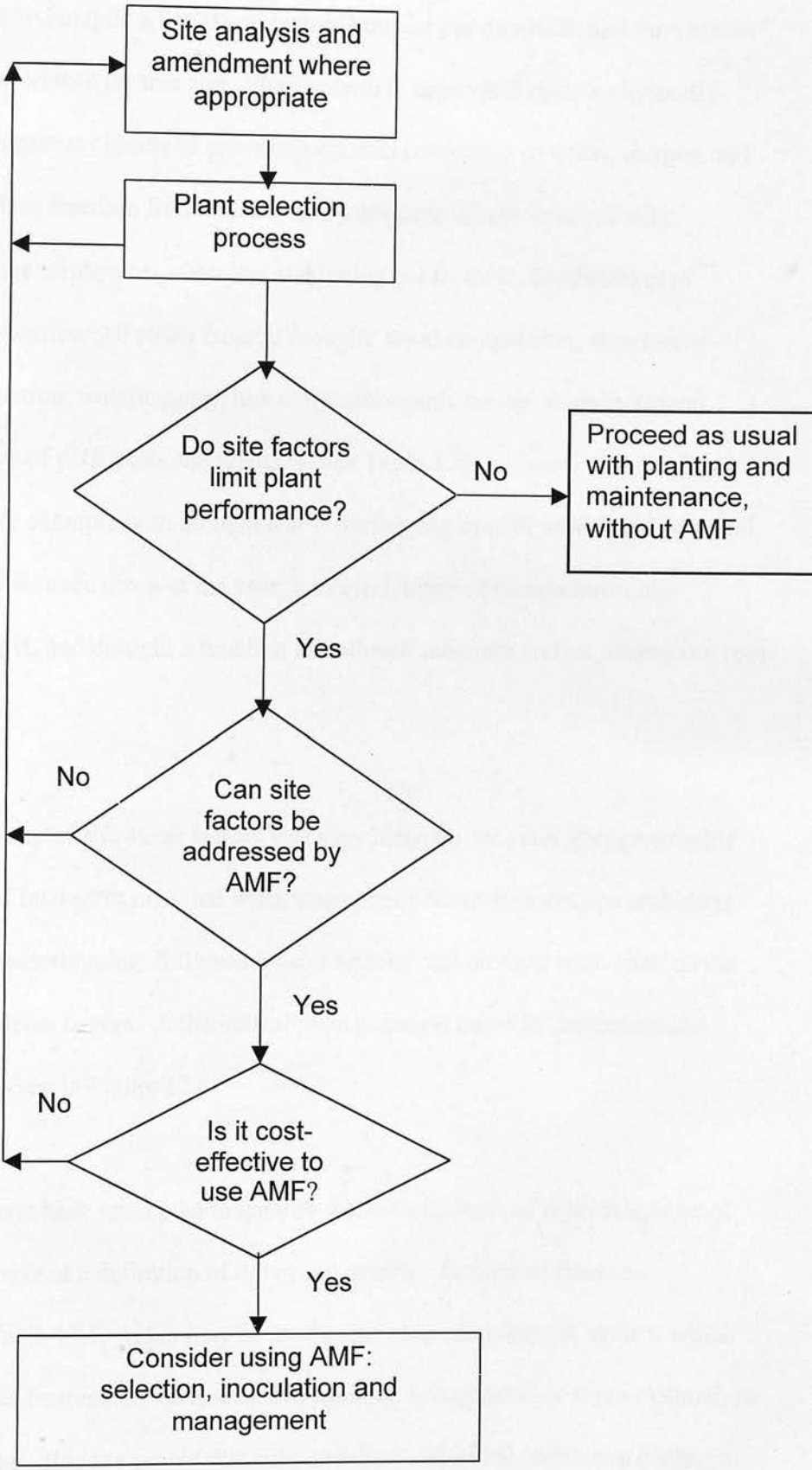
12.2.3. *Landscape model*

This approach combines aspects of the physiological model suggested by Fitter [238] with the economic approach (response iii) of Miller *et al* 124 supplemented by decision theory, and is summarised in Figure 12.3. The model assumes that site evaluation, amendment and plant selection processes have been completed, and that site factors are expected to reduce plant performance. Information for the model includes:

- an assessment of site stress factors (expressed as percentage of optimal) which are expected to restrict plant and AMF performance
- a definition of acceptable plant performance for that site (expressed as percentage of optimal)
- the probability of plant benefits from AMF in those site conditions.

This information is then combined with the cost of inoculation/mycorrhizal management, and the cost of plant failures and replacement.

Figure 12. 3. An AMF Decision Strategy for Landscape Management.
(Adapted from Dodd andThompson,[121] ; Hitchmough[125]).



12.3. DERIVATION OF VARIABLES FOR A LANDSCAPE MODEL

12.3.1. Site factors

The first stage is to compile a list of soil factors limiting plant growth, and then assess their relative importance for that site. Plant growth is dependent upon a physically stable soil environment capable of providing optimal conditions of water, oxygen and nutrients, as well as freedom from toxicity and pathogens. These requirements encompass a range of physical, chemical and biological factors. Bradshaw *et al* [12] identified the following soil stress factors: drought; weed competition; structure, including compaction; waterlogging; low nutrients/organic matter; toxicity (metal, organic, extremes of pH); pests and diseases (See Table 3.2). Several of these factors are correlated, for example both drought and waterlogging may be associated with soil compaction at difference times of the year; low availability of certain nutrients associated with pH, and drought a function of both soil moisture and an inadequate root system.

The relative severity of site stress factors will vary between sites and also geographic locations. It is of interest to note that when a sample of Scottish landscape architects were contacted, waterlogging, followed by soil toxicity and drought were cited as the most important stress factors. A theoretical plant response curve to environmental stress factors is given in Figure 12.4.

Various researchers have attempted to identify the critical limits of plant tolerance of soil factors, to arrive at a definition of urban soil quality. Several of these are summarised in Table 12.1, which may be used to develop a benchmark against which the severity of site factors may be assessed as optimal, sub-optimal or supra-optimal, to plant performance. It is noticeable that only Craul's [12] definition includes a biological

factor, namely earthworms; and that none consider an assessment of the presence of beneficial or pathogenic rhizosphere micro-organisms. The site stress factors, once identified and evaluated, may then be ranked in order of expected restriction of plant performance.

While it is known that plants vary both inter and intra-specifically in their edaphic and climatic requirements, this model makes the rather broad assumption that 'landscape plants' comprise a homogenous palette of plants with similar environmental tolerances, mycorrhizal status and dependency.

Figure 12. 4. Theoretical plant response to environmental factors

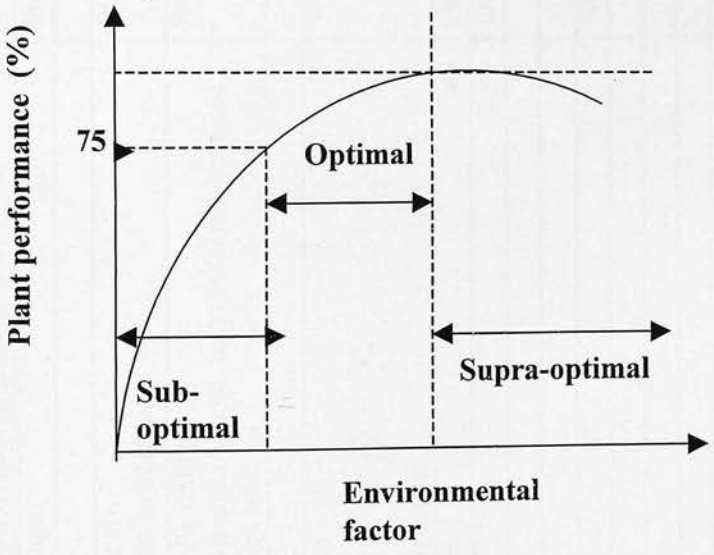


Table 12.1. Urban soil quality for landscape plants

Parameter	Optimal range	Significance	Source
1. Physical			
Bulk density	<1.5 to 1.7 g cm^{-3} Depending on soil texture	Water, oxygen, mechanical impedance	Moffat & Bending [247] Huinink [248]
Depth	Minimum 500-1000 mm Depending on soil material, up to 2000 mm required on some stony soils	Rooting depth	Moffat & Bending [247]
	>1500		Craul [18]
Texture	20-75% sand 5-60% silt 5-30% clay >4% organic matter	Water, oxygen, ability to tolerate trafficking	Volker & Dinsdale [249]
	<40% stones	Porosity, stability	Moffat & Bending [247]
Air capacity	>10%	Oxygen	Craul [18]
2. Chemical			
Soil pH	3.5-8.5	Nutrient availability/toxicity	Moffat & Bending [247]
	5.5-7.8		Volker & Dinsdale [249]
	7-7.5		Craul [18]
	4.8-7.5		Huinink [248]
Nutrients	>0.2% N (Kjeldahl) >45 ppm P Extractable >240 ppm K Extractable >80 ppm Mg Extractable	Essential for plant growth	Volker & Dinsdale [249]
Conductivity	<2000 µS cm^{-3} <1500	Toxicity	Moffat & Bending [247] Huinink [248]
Toxicity (Heavy metal, organic)	Not exceeding ICRL action trigger contaminant concentrations	Toxicity	Moffat & Bending [247]
3. Biological			
Earthworms	>200 m^{-3}	Soil health	Craul [17]

12.3.2. Plant performance

How can an economic value be attached to any of the potential benefits accrued from inoculation with AMF? Few landscape taxa are grown for biomass production, and so yield has little relevance. Similarly, excessive growth beyond the establishment phase cannot be viewed as beneficial as it may incur additional maintenance costs in pruning and thinning. Plant performance on site is a combination of survival, vigour and visual condition. It is suggested that a critical threshold exists at which practitioners might consider changing their course of action in order to improve plant performance. This will be a function of the extent to which plant performance fulfils the original design intentions for that site.

Lay and Meissner [250] suggested an objective method for assessing the performance of amenity plantings based on health and vigour criteria scales. Plants were scored on a scale of 0 to 5: where 0 indicated plant death, and 5 that greater than 75% of optimum growth (vigour) or less than 25% leaf damage (health) were achieved. These scale ratings, which depend on some knowledge of optimum growth, could then be analyzed statistically.

Bradshaw *et al* [12] assigned a monetary value to the cost of purchasing and planting stock based on Spon's Landscape and External Works Price Book [252] (updated annually) to assess the financial implications of tree failure. Mention was also made of the Arboricultural Association Amenity Valuation of Trees (1990), the less tangible psychological value of trees in the environment, and the contribution of trees to noise and urban pollutant reduction. Although poor plant performance will have an impact on the aesthetic and functional value of the site, this is difficult to assess in monetary terms. At present it is probably more realistic to consider only the costs of replanting to

achieve the critical threshold of plant performance appropriate to that planting site. It is expected that on a prestigious and high impact scheme, the critical threshold for plant performance would be considerably higher than on a less prestigious scheme.

12.3.3. Likelihood of AMF benefits to plant performance

The next stage is to estimate the likelihood that AMF will benefit plant performance above the critical threshold of plant performance. This is a determined by the 'mycorrhizal triangle' of soil-plant-AMF factors:

- AMF genotype: adaptation soil environmental conditions (physical, chemical and biological); infectivity (ability to infect host); effectiveness (ability to improve plant performance)
- Plant genotype: adaptation to soil environment; dependency on AMF (susceptibility to infection, extent to which it can satisfy requirement for soil resources without AMF)
- Soil environment: physical, chemical and biological characteristics; how tolerated by plant and AMF

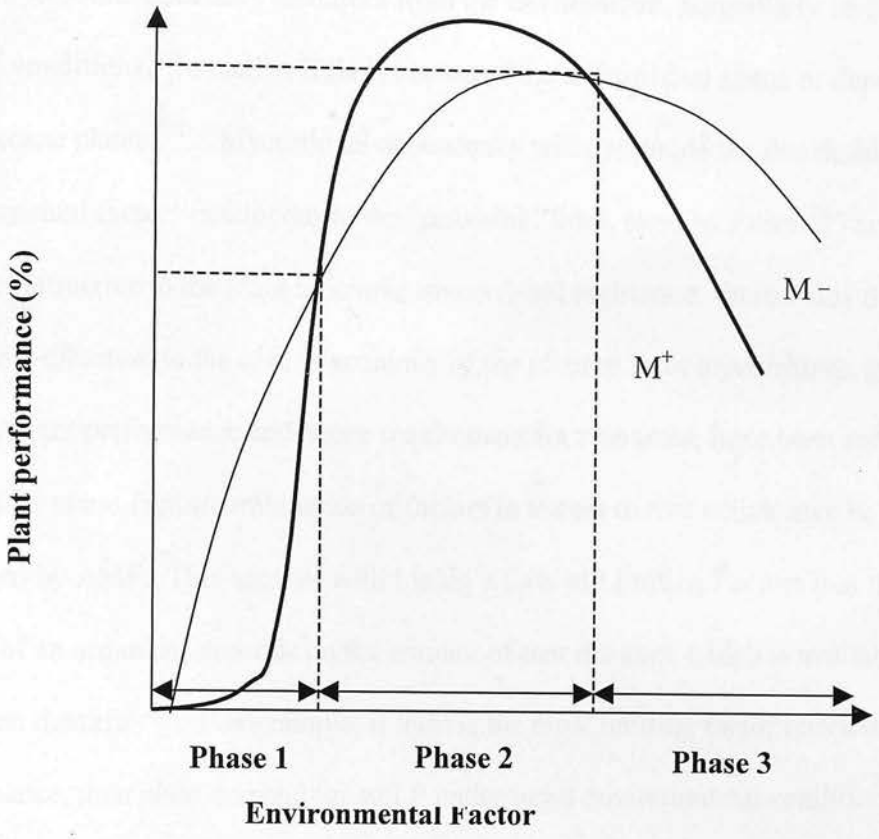
This stage assumes that all AMF have the same environmental tolerances, host preferences, and ability to compete with other rhizosphere micro-organisms (indigenous AMF, mycorrhiza helper bacteria, plant and AMF pathogens and predators). Although this is known not to be the case – over 130 taxa of AMF have been recognised, distributed throughout most terrestrial ecosystems – in reality only a few isolates are commercially available.

It is generally assumed that mycorrhizal fungi may have a broader tolerance of extremes of environmental conditions than their host roots. Therefore it is likely that AMF will

be beneficial to plant performance when the fungi are above their own critical threshold of environmental tolerance, but that conditions are sub-optimal for the plants. Peat and Fitter [103] and Klironomos [95] presented evidence that AMF may extend the ecological range of some plant taxa. However, Don Marx of Plant Health Care (pers. comm.) has suggested that when trees are planted into soils beyond their normal pH tolerance, then abnormal root growth may occur, which is less susceptible to mycorrhizal formation. Fitter [238] estimated that AMF consume 10% of root carbohydrate, and so it is assumed that when environmental factors are less than 10% of optimum for plant performance (the zero-sink threshold), it will be more cost-effective to the plant not to form mycorrhizal associations.

A second hypothetical response curve is proposed (Figure 12.5) which indicates several stages of mycorrhizal formation:

Figure 12.5. Theoretical plant-AMF response to environmental factors.



- **Phase 1** : Below environmental threshold of plant requirements (approximately 10%) AMF formation is not cost-effective to the host plant. Hence the non mycorrhizal curve (M^-) has a higher level of plant performance.
- **Phase 2** : Above the minimum threshold of plant requirements, to optimal conditions, it is more cost-effective to the host plant to form AMF. Hence the mycorrhizal response curve (M^+) has a higher level of plant performance than non-mycorrhizal (M^-).
- **Phase 3 :** As optimal conditions for plant host are reached AMF may become commensal (neither beneficial nor non-beneficial) and so the mycorrhizal (M^+) and non-mycorrhizal (M^-) responses intercept. However as supra-optimal conditions are reached, AMF may become non-beneficial or even parasitic. After this point the mycorrhizal (M^+) curve has a lower level of plant performance than the non-mycorrhizal curve (M^-).

Mycorrhizal dependency is a function of plant and fungus genotype, and the ability of the plant to obtain necessary resources from the environment, particularly under sub-optimal conditions. In reality, little is known of the mycorrhizal status or dependency of landscape plants [252]. Mycorrhizal dependency will determine the threshold of environmental factors (analogous to the 'zero-sink' limit, cited by Fitter [238]) at which it becomes attractive to the plant to invoke mycorrhizal assistance. Below this threshold it is not cost-effective to the carbon economy of the plant to form mycorrhizas, generally because plant performance and hence requirement for resources, have been reduced by some other stress factor/combination of factors in excess of that which may be addressed by AMF. This accords with Liebig's Law of Limiting Factors that the final growth of an organism depends on the amount of that resource which is available to it in minimum quantity [239]. For example, if light is the most limiting factor restricting plant performance, then plant demand for soil P under those environmental conditions may be relatively small, compared to the carbon cost of additional AMF-mediated uptake. Kendle [19] suggested that, under some conditions, plants are too stressed, by a combination of plant and environmental factors and therefore carbon deficient, to form mycorrhizal associations. This is corroborated by Don Marx (Plant Health Care) and also Ted St John (Tree of Life) (pers. comm.) who indicated several scenarios in which AMF might not be beneficial:

- extremely fertile soils
- frequently irrigated sites where 'water' roots might form
- soils with more than 15% organic matter, as this may encourage anaerobic respiration
- shade-intolerant trees grown in heavy shade, for example covered sports complexes, some under-canopy plantings
- high pesticide/fungicide applications

- extreme soil pH.

The high carbon cost of maintaining the AMF on a less than optimal site is one interpretation of the negative effects of AMF inoculation on cherry observed in the field experiment (Chapter 10). However, in reality it is extremely difficult to obtain data on the stress limits on AMF formation, perhaps because of a reluctance to publish negative results, or a difficulty in uncoupling the association under controlled conditions.

Recently, an Internet database site (hosted at HRI Efford, and funded by the Ministry of Agriculture, Fisheries and Food) has been set up to compile mycorrhizal dependency data on a range of cultivated plants. While extremely limited at present, the site offers three definitions of mycorrhizal dependency which suggest a potential way forward:

i) Simple mycorrhizal field dependency (SMFD) [253] expressed as a percentage where, for whatever parameter has been assessed:

$$\text{SMFD} = \frac{\text{parameter with AMF} - \text{parameter without AMF}}{\text{Parameter with AMF}}$$

The maximum dependency is 100%.

ii) P-related dependency, where dependency has been determined at fixed points of P.

iii) Complex dependency, where the factors controlling dependency are modeled (using regression formula). This enables predictions to be made

by interpolation, and can be modified as new information becomes available.

At present however, with the exception of the few landscape taxa that have been studied under field conditions [73, 244, 79, 80, 81] and the field trial carried out in Chapter 10, mycorrhizal dependency can only be approximated using subjective probabilities of benefits, gauged from expert/scientists' opinion.

12.3.4. *Economic cost-benefits*

The final stage of the model is to arrive at an economic appraisal of the cost of replanting to achieve the critical level of plant performance, the cost of inoculation and management of AMF, and the likely benefits in terms of improve performance and reduction in inputs such as fertilizers.

i) Planting

According to Spon's Landscape and External Works Price Book [251], the total cost of planting 100 broad-leaved transplants is £670. (Table 12.2.) This size of plant material has been chosen as it approximates with that used in most experimental studies. The total cost includes supply of plants, ground preparation, digging planting holes, planting, backfill, staking and tree guard, fertilizer, herbicide and pesticide, as well as an allowance of 10% beating up of original planting. For a relatively cheap tree species like rowan with a unit cost of £0.26, the cost of stock represents less than 5% of the total planting costs. If all the plants died and had to be replaced, replacement costs are estimated at 50% of original total planting costs. This is on the assumption that although plants would have to be re-purchased, stakes and guards would not, but that

there would still be labour involved in clearing away dead trees, re-planting and re-staking.

Table 12.2. Planting and inoculation costs for 100 bare root transplants. (Spons's Landscape and External Works Price Book [251])

	Average cost (£)	% of cost
1. Original planting scheme		
Purchase of stock	26.0	4.0
Ground preparation, planting and maintenance, 10% beating up	644.0	96.0
Total	670.0	100.0
2. Replanting[1]		
Purchase of stock	26.0	
Planting, fixing stake and tree guards	309.0	
Total	335.0	50.0
3. Inoculation[2]**:**		
Purchase of 'Mycorrtree' root dip	16.0	
Application	1.4	
Total	17.4	2.6

[1] Assuming 100% failure
[2] Prices obtained from inoculum companies (see Table 3).

ii) Inoculation

A selection of inoculum suppliers were contacted May 1999 for details of inoculum products and costs. These are given in Table 12.3. The products differ in species composition, the number of effective AMF propagules and recommended application rate. However, it would appear that prices ranged from £0.02 to £0.05 per 100 ml pot for compost incorporation (slightly larger than the average plant cell), to £0.16 per whip for root dipping prior to planting. Assuming that compost incorporation has a negligible cost, but that the cost of applying a root dip is an additional £0.014 per plant (based on Spon's 'Alginure' Root Dip £0.024, less £0.009 price of product), then the total cost of inoculating a bare root would be approximately £0.18. This represents an additional cost of 35% when considered on a plant material only basis, but less than 3% when total planting costs are considered.

Table 12.3. Inoculum products and pricing details (May 1999).

Product	Species Present	Use	Application Rate	Unit Cost	Cost per plant
'Vaminoc' MicroBio Ltd. UK	G.*mosseae*, *G. caledonium, G. fasciculatum* (Not compatible with peat, recommend single endophyte *G. intradices*)	Compost incorporation / Direct placement	2.5 g per 100ml pot	£10 / kg	£0.04
VAM Nursery Media Mix 'MycorTree Root Dip' Plant Health Care, UK	*Entrephospora columbiana; G. etunicatum; G. clarum* , *Glomus spp.* Also contains : *Bacillus* spores; *Pisolinthus tinctorius;* Yucca plant extract; Sea Kelp extract; Humic acids.	Compost incorporation Root tip for bare root plants	0.36 g per 100 ml pot 85g per 100 trees	£450/10 kg box £16 per 85g	£0.02 £0.16
'Endorize' Biorize France	*G. mossese, G. fasciculatum, Glomus* sp., *Sclerocystus* sp	Compost incorporation.	5% (V/V) = 5ml per 100 ml pot	£10 / l	£0.05
'Mycorise' Premier Tech Canada	*G. intradices*	Seedling Nursery bed Compost incorporation	2.5 g/ plant 2 kg/30-60m^2 70 l m^{-3}	£1.41 /l	£0.04 - -
'VAM80' Tree of Life Nursery. California, USA	*G. intradices*	Land reclamation Agricultural soils Nursery seedling Compost incorporation	150 l ha^{-1} 75 l ha^{-1} 1 ml per plant -	£4.25 /l	- - £0.04

iii) Management

Although there is some evidence that mycorrhizal plants allow inputs of fertilizer and pesticides to be reduced, in reality there is little practical guidance for the landscape profession. Fertilizer costs (usually cited as the input most likely to be reduced by the use of AMF) range from £19 for at an application rate of 35g m^{-2}, to £47 at an application rate of 150g m^{-2}. Therefore fertilizer costs would comprise 3 to 7% of total planting costs. There may also be additional costs involved in managing mycorrhizal plants, for example greater care in handling plants, reduced soil cultivation, use of more compatible pesticide products. These aspects were reviewed in Section 3.5 (See Table 3.5 for summary of AMF management practices). However, at this stage of the development of a model, AMF management costs are ignored.

Thus at the end of this stage we can begin to explore a landscape model based on the following assumptions which may be amended as more information becomes available :

1. Landscape plants are a homogenous group of plant taxa, with broadly similar environmental tolerances and the same mycorrhizal dependency.
2. Plant performance can be quantified as an amalgam of survival, vigour and visual condition (expressed as a percentage) with a critical threshold for each planting scheme, at which it is desirable to consider changing the current course of action.
3. Site stress factors can be expressed as a percentage of optimum plant growth requirements, and that the relative importance can be ranked.
4. AMF are a homogenous group of fungi with the same environmental tolerances and ability to associate with landscape plants.

5. It is assumed that no significant populations of indigenous AMF are present on site, and that the plants arrive without AMF.
6. In the absence of verifiable data, mycorrhizal dependency for a range of environmental conditions can be assumed from expert opinion.
7. Costs are based on replacement planting costs and the cost of inoculation / mycorrhizal management.

12.4. EXPLORATION OF THE LANDSCAPE MODEL

Two approaches are considered to the decision making process when there is uncertainty in the outcome: a simple decisional matrix, and a decision tree [254].

12.4.1. Decisional matrix

Assume that the landscape practitioner has the choice of using AMF (M+) of not using AMF (M-), and that plants can either survive or fail. There is a probability (p1) of plant survival using M+, a probability of plant failure (1-p1) using M+, a probability of plant survival using M- (p2) and a probability of plant failure using M- (1-p2) as follows:

Probability Matrix

	Survive	**Fail**
M+	P1	(1 – P1)
M-	P2	(1 - P2)

Cost Matrix

Survive	**Fail**
688	1032
670	1005

Then for decision:

$M+ \quad 688\,(p1) + 1032\,(1\text{-}p1) \rightarrow 1032 - 344p1$

$M^- \quad 670\,(p2) + 1005\,(1\text{-}p2) \rightarrow 1005 - 335p2$

Then for action M+ to be the preferred option:

$$1032 - 344p1 < 1005 - 335p2$$

$\longrightarrow$ $27 < 344p1 - 335\ p2$

if the probability of plants surviving without mycorrhizal inoculation (p2) = 0.5:

$194.5 < 335p1$

$\longrightarrow$ $p1 < 0.58$

Therefore, for this combination of costs and probabilities, inoculating trees (M+) is cost-effective if p1 >0.58. A small increase of 0.08 in the probability that survival will be increased, is sufficient to warrant action M+.

An alternative approach might be to use Subjective Expected Utilities (SEU) where practitioners assign a subjective, non-monetary utility to a particular outcome.

Assuming that:

- p2 (all plants survive without inoculation) is the best possible outcome (SEU = 1)
- 1-p1 (all plants die after inoculation) is the worst possible outcome (SEU = 0)
- p1 (plant survival with M+) might have a slightly lower SEU of 0.8, compared to M- because of the cost of inoculation.
- 1-p1 (plant failure without inoculation M-) might have a slightly higher SEU compared to failure after inoculation (M+) , because no additional inoculation costs have been incurred.

Probability Matrix

	Survive	**Fail**
M+	P1	(1 – p1)
M-	P2	(1 - p2)

SEU Matrix

Survive	**Fail**
.8	0
1	.1

It would be worthwhile using M+ where :

$$0.8\ p1 > p2 + 0.1 + (1 - p2)$$

$$\longrightarrow \quad 0.8\ p1 > 0.9\ p2 + 0.1$$

if p2 = 0.5 (50% probability plant success without mycorrhiza)

$$0.8\ p1 > 0.45 + 0.1 > 0.55$$

$$\longrightarrow \quad p1 > 0.55/0.8 > 0.69$$

Using this scenario, then using M+ is considered cost-effective if p1 >0.69. An increase of 0.19 (0.69 – 0.50) in the probability that survival will be increased, is therefore the threshold for perceivable benefits, and is sufficient to warrant action M+.

12.4.3. Decision tree

A more elaborate decision tree may be constructed (Figure 12.6). Here, for each level of environmental conditions (sub-optimal, optimal or supra-optimal), the probability of plant performance being high, moderate or low is estimated both with (M+), or without (M-) mycorrhizal management. Each course of action is then given a probability (p1…..p18), a cost (for example Table 12.2) or utility (SEU).

As part of the initial site assessment, the level of environmental conditions, are assessed by the landscape practitioner. The probability of whether plant performance will be high, moderate or low are estimated from 'expert' opinion at present. However as more data becomes available from future research, actual figures of plant performance may be used. The cost or utility of each course of action, would also be supplied by the practitioner.

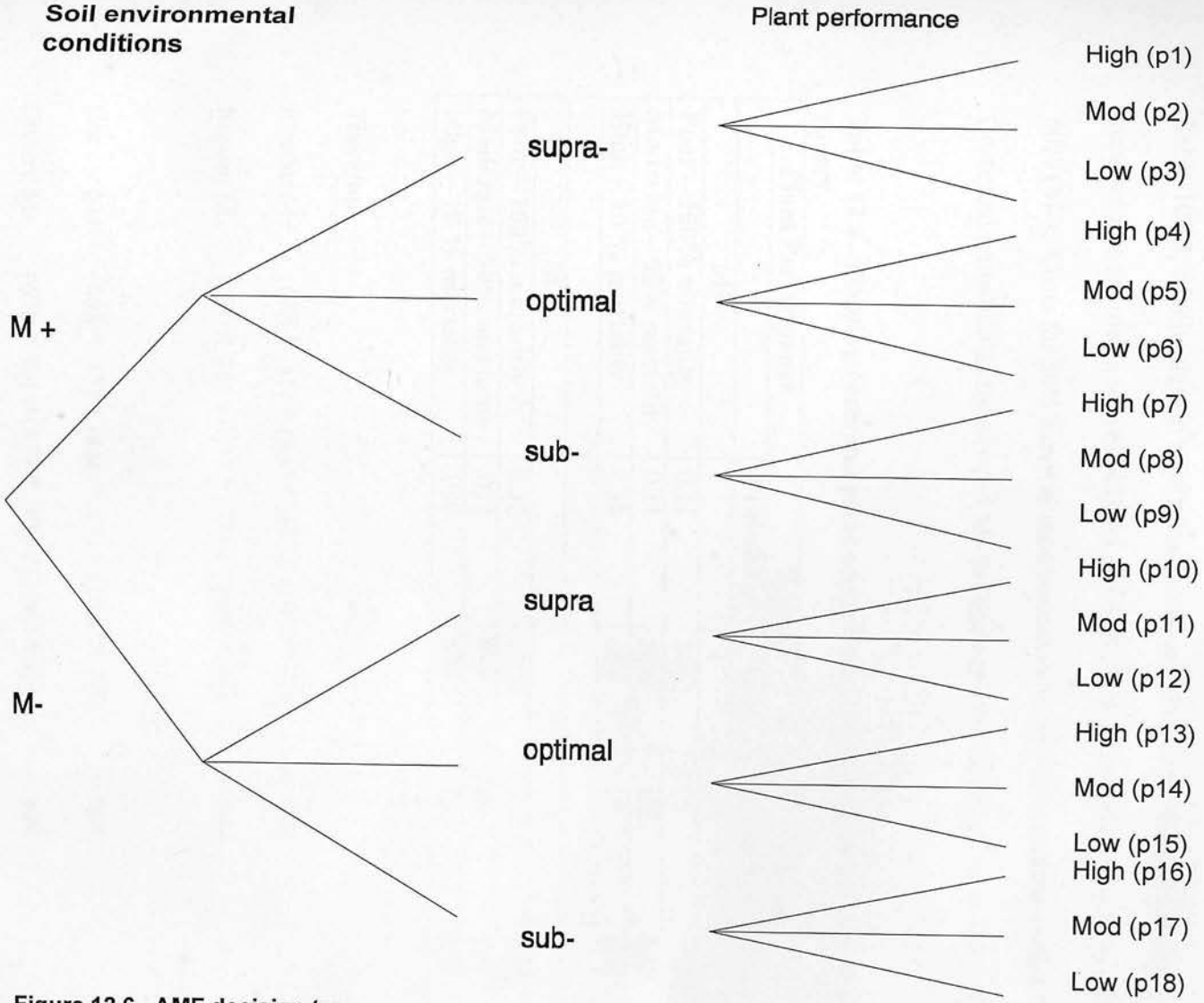


Figure 12.6. AMF decision tree.

To decide which course of action to adopt, the practitioner would multiply the cost/utility of each outcome by the probability of a particular level of plant performance. The products of each branch are then summed across each of the courses of action, and the option chosen which appears to minimise costs or maximise utility.

For example, using costs from Table 12.2, and probabilities of survival derrived from Table 10.13, which relate to survival in rowan and cherry on Stankards Bing. The survival rate for rowan was 62% (M+) and 64% (M-); for cherry it was 35% (M+) and 60% (M-). Costs for 50% survival are assumed to be 50% of complete re-planting. Costs and probabilities for M+ and M- in cherry and rowan are given in Table 12.4.

Table 12.4. Planting costs and plant performance probabilities for rowan and cherry.

Plant Performance	Rowan		Cherry	
	Probability	Cost	Probability	Cost
M+				
Poor – 100% mortality	0.31	1032	0.17	1032
Moderate – 50% mortality	0.31	860	0.18	860
High – 10 % mortality	0.38	688	0.65	688
M-				
Poor – 100% mortality	0.32	1005	0.30	1005
Moderate – 50% mortality	.032	837	0.30	837
High – 10 % mortality	.036	670	0.40	670

Therefore:

Rowan M+ (688 * .31) + (860 * .31) + (1032 * .38) = 872

Rowan M- (670 *.32) + (837 * .32) + (1005 *.36) = 844

Cherry M+ (688 * .17) + (860 * .18) + (1032 * .65) = 943

Cherry M- (670 *.30) + (837 * .30) + (1005 *.40) = 854

Using this data, it appears that for rowan and cherry the lowest cost outcome is associated with M-. The difference between the two outcomes is marginal for rowan (28/844 or 3%), while more substantive for cherry (89/854 or 10%).

The outcome might also be estimated using SEU, where a practitioner assigned a value to each of the levels of plant performance, for example:

5 for high plant performance

2 for moderate plant performance

0 for poor plant performance

Rowan M+	(5 * .31) + (2 * .31) + (0 * .38)	= 2.17
Rowan M-	(5 *.32) + (2 * .32) + (0 *.36)	= 2.24
Cherry M+	(5 * .17) + (2 * .18) + (0 * .65)	= 1.21
Cherry M-	(5 *.30) + (2 * .30) + (0 *.40)	= 2.10

As these are Subjective Expected Utilities, we seek to maximise the sum for the preferred decision. Again, the figures are in the same direction, with M- being the preferred option for both rowan and cherry. However it should be noted that these probabilities refer to only one experiment on one site, and that as more data becomes available on plant performance from mycorrhizal experiments, these probabilities might be given with greater precision.

12.5. CONCLUSION

In this chapter a conceptual approach to developing a mycorrhizal decision model has been presented, which incorporates elements of plant physiology, economic cost-benefits and decision theory. At present the model make many assumptions about the 'mycorrhizal triangle' of plants, AMF and soil environmental conditions. To some extent environmental tolerances and response to mycorrhizal inoculation may be estimated from 'expert' scientific opinion, however it is anticipated that as more data from field experiments enters the public domain, then the probabilities on which the model is based may be estimated with greater precision. At present the field functioning of mycorrhizas, despite appearing to be a promising technology, is beset with uncertainty and assumptions. It is hoped that, as with most landscape management practices, a creative approach to plant establishment, underpinned by replicated scientific trials and a wider dissemination of results, will provide a more certain basis for future recommendations to practitioners.

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APPENDIX ONE. THE MAIN TREE SPECIES NATIVE TO SCOTLAND.

(Adapted from Soutar [4]; Harley and Harley [66]; Grime *et al.*[166])

Family / Species	Suitable Site Conditions						Myc.	Strategy
	Wet soils	Light, dry soils	Heavy soils	Acid soils	Neutral/ alkaline	Exposed sites		
AQUIFOLIACEAE								
Ilex aquifolium			X		X	X	AM (ECM?)	S-C
BETULACEAE								
Alnus glutinosa	X				X		ECM/ AM [b]	S-C
Betula pendula		X	X	X	X	X	ECM	S-C /C
Betula pubescens	X	X	X	X	X	X	ECM	S-C /C
CORYLACEAE								
Corylus avellana		X	X		X	X	ECM	S-C
FAGACEAE								
Quercus petraea		X		X	X	X	ECM	S-C
Quercus robur	X		X	X	X	X	ECM	S-C
OLEACEAE								
Fraxinus excelsior		X	X		X		AM (ECM?)	C
PINACEAE								
Pinus sylvestris		X		X		X	ECM	-
ROSACEAE								
Crataegus monogyna		X	X	X	X	X	AM (ECM?)	S-C
Prunus avium			X		X		AM (ECM?)	S-C
Prunus padus	X				X		AM (ECM?)	S-C
Prunus spinosa	X	X	X		X	X	AM	S-C
Sorbus aucuparia		X		X		X	AM (ECM?)	S-C
SALICACEAE								
Populus tremula	X	X	X		X	X	ECM /AM	S-C
Salix alba	X				X	X	ECM /AM	-
Salix caprea	X		X		X	X	ECM /AM	-
ULMACEAE								
Ulmus glabra			X		X	X	AM	C

[a] S-C : Stress tolerant competitor; C: Competitor.

[b] Also forms associations with the actinomycete *Frankia* spp.